Molecular characterization and antimicrobial resistance of scarlet fever and invasive *Streptococcus pyogenes* in England and Wales, 2014

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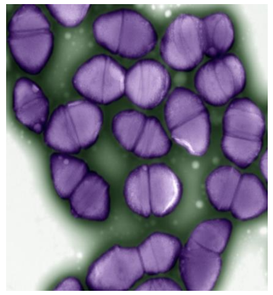
INTRODUCTION



- *Streptococcus pyogenes* (group A streptococcus, GAS) causes: impetigo, puerperal sepsis, necrotising fasciitis, tonsillitis, scarlet fever, pneumonia, septicaemia and meningitis.
- An unprecedented increase in the number of scarlet fever (SF) cases was reported in England during 2014 (1).
- **AIM – to characterise by phenotypic antimicrobial susceptibility and known molecular mechanisms of resistance GAS isolates from patients with SF and invasive infection (iGAS) to determine whether the SF increase was attributed to a single clonal lineage with increased resistance.**

METHODS

- Isolates included were referred during April-June 2014:
  - 308 from patients with SF (throat swab);
  - 237 from patients with iGAS.
- *emm* gene sequence typing (derived from genomic sequences).
- MICs by agar dilution and/or E-test (EUCAST guidelines): erythromycin, penicillin, ciprofloxacin, rifampicin and tetracycline.
- Genomic sequence data by Illumina methodology.
- Bioinformatic analysis performed against a locally-curated database of resistance determinants.

EMM GENE TYPING

- Isolates were polyclonal - 25 *emm* types identified.
- *emm* 3 represented 36% of total isolates, followed by *emm* 1, 12 and 89.
- Similar diversity and distribution of *emm* types for SF and iGAS.
- All iGAS *emm* types were also found in SF.

emm type	iGAS (%)	SF (%)
3	27	43
1	23	9
89	10	3
12	7	17
28	6	4
6	4	6
others (*emm* 2,4, 5, 9, 11, 18, 22, 44, 53, 58, 73,75, 76, 77, 81, 82, 87, 90 and 94)	20	19
Total	237 isolates	308 isolates

RESULTS

PHENOTYPIC SUSCEPTIBILITY AND GENOMIC DATA

Erythromycin

- 3.3% (n=18) resistant isolates – similar percentage observed by GAS bacteraemia data (2).
- The *erm*(B), *mef*-(A), *mef*-(E), *msr*-(D) genes as well as mutations in the L4 and L22 and 23S regions were detected in genomic sequence data.
  - *erm*(B): in 8 resistant isolates, absent from all susceptible.
  - *erm*(B) positive were high level resistant (MIC >16 mg/L).
  - *mef*A: in 2 resistant isolates; and in 5 susceptible isolates - ? Gene expression/efficiency factor.
  - *Mef*-(E) & *msr*-(D) in 1 and 6 susceptible isolates, respectively.
  - Mutations in L2 and L4 were not specifically associated with resistance.
  - 44 mutations detected in the 23S rRNA gene; 13 in resistant isolates; 4 were exclusive.
  - 2 isolates had *erm*(B) & a mutation in the 23S:129:C-T.
  - 2 isolates had *erm*(B) & a mutation in the 23S:305:C-Y.
  - These mutations were not found in any susceptible strains. Association with resistance cannot be excluded.
  - 1 resistant isolate no known genes conferring resistance, but had mutations 23S 2061:A-R & 2702:C-T.
  - Neither of these mutations were found in any susceptible strains. Definitive association with resistance requires further investigation.
- Mutations or genes exclusive to resistant isolates were not identified in 7 of the 18 resistant isolates, further investigation is underway.

Antibiotic tested (Eucast breakpoints)	Resistant		Susceptible		Fisher's exact test
	SF	iGAS	SF	iGAS	*p* value
Erythromycin (0.5mg/L)	6	12*	296	231	0.095
Ciprofloxacin (Eucast ECOFF 1mg/L)**	nil	1	308	236	0.43
Tetracycline (2mg/L)	3	17	305	220	0.0001
Rifampicin (0.5mg/L)	nil	nil	237	308	1
Penicillin (0.25mg/L)	nil	nil	237	308	1

*9 isolates high level resistant (MIC>16mg/L)
**Ciprofloxacin MICs for 214 isoaltes (111iGAS and 103SF) 1mg/L, remaining 330 isolates <=0.5mg/L

Tetracycline

- 3.6 % resistant isolates; lower than in GAS bacteraemia susceptibility data (2), but similar for iGAS only (14%).
- 17 resistant isolates had *tet*(M), and 2 *tet*(O) – both genes encode ribosomal protection proteins.
- *tet*(M) and *tet*(O) were absent in all tetracycline susceptible isolates (n=525).
- Neither gene was found in one resistant *emm* 5 iGAS isolate (MIC 8mg/L) and further investigations are underway.

Ciprofloxacin

- 4 mutations in *par*C and 1 in *gyr*A were identified. None were exclusive to non-wild type strains. A single non-wild type isolate (MIC 2mg/L) with parC 305:C-Y mutation was identified, but also identified in 27 isolates with MIC below the ECOFF (1mg/L).

Rifampicin and penicillin:

- Isolates universally susceptible to rifampicin and penicillin, despite *rpo*B gene and penicillin-binding protein gene mutations, respectively.

SUMMARY

- Isolates were fully susceptible to penicillin and rifampicin & 1 isolate was non-wild type to ciprofloxacin ECOFF.
- Macrolide and tetracycline resistance were observed in 3.3% and 3.6 % of isolates, respectively.
- *erm*(B) associated with high level erythromycin resistance.
- Further analysis required to confirm the point mutations in 23sRNA gene putatively associated with macrolide resistance and also the expression of *mef*A.
- Further work is underway to identify single nucleotide polymorphisms/genes conferring resistance (7 erythromycin-resistant isolates).
- tetracycline resistance was associated with *tet*(M) and *tet*(O) & was significantly higher in iGAS isolates versus SF
- SF and iGAS isolates were polyclonal with susceptibility data within expected ranges, indicating SF could not be attributed to a single lineage with increased resistance

References: 1- *Health Protection Report* Vol 8 No. 11 **–** 21 March 2014;
2-BSAC Bacteraemia Resistance Surveillance Programme, ww.bsacsurv.org, accessed on 16th March 2016