

Guiding *Neisseria gonorrhoeae* management by molecular detection of ciprofloxacin resistance by ResistancePlus GC assay (SpeedX)

Seb Cotton, Michelle Etherson, Naomi Henderson, Jill Shepherd, Kate Templeton. Royal Infirmary Edinburgh, Edinburgh, United Kingdom. Seb.Cotton@nhslothian.scot.nhs.uk, Kate.Templeton@nhslothian.scot.nhs.uk

Aim

To evaluate the clinical performance of the ResistancePlus GC assay compared to the in-house PCR and antimicrobial susceptibility results for ciprofloxacin.

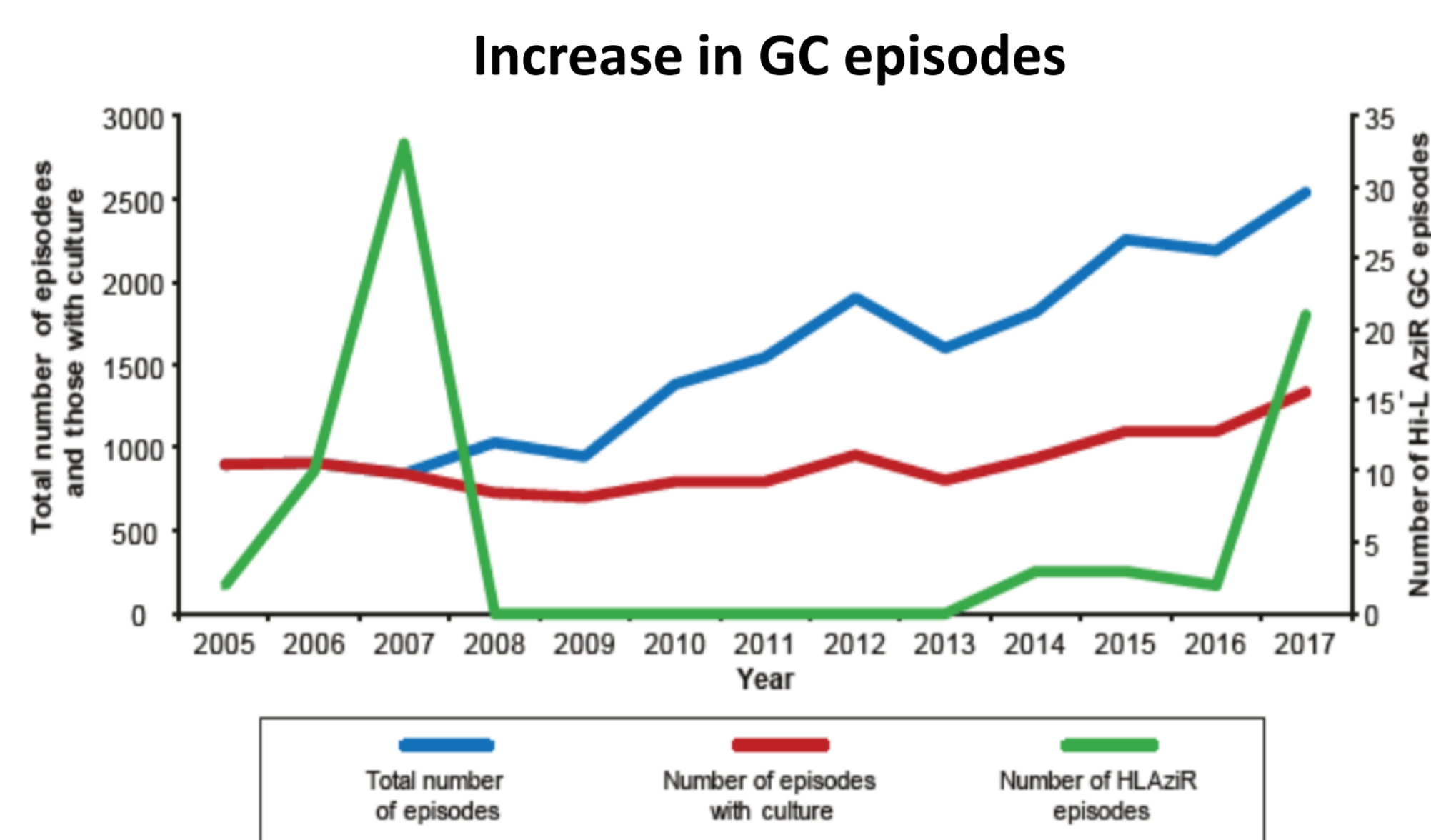
Introduction

Gonorrhoea is one of the most prevalent sexually transmitted infections and there is a growing concern due to the emergence of multi-drug resistance developed in the causative agent *Neisseria gonorrhoeae* (GC).

In Scotland there has been an increase in GC episodes and high-level azithromycin episodes in particular shown in graph 1.

Recommended treatment is with ceftriaxone although new guidelines from BASHH suggest treatment with ciprofloxacin can be used if susceptibility is known.

ResistancePlus GC assay is a rapid molecular test to detect the S91F mutation in DNA gyrase. It could provide ciprofloxacin susceptibility knowledge prior to start of treatment and decrease the turnaround time from current culture methods (7 days).



Graph 1: Total number of GC episodes, High level azithromycin episodes and episodes with a culture from 2005-2017 in Scotland (GASS 2018).

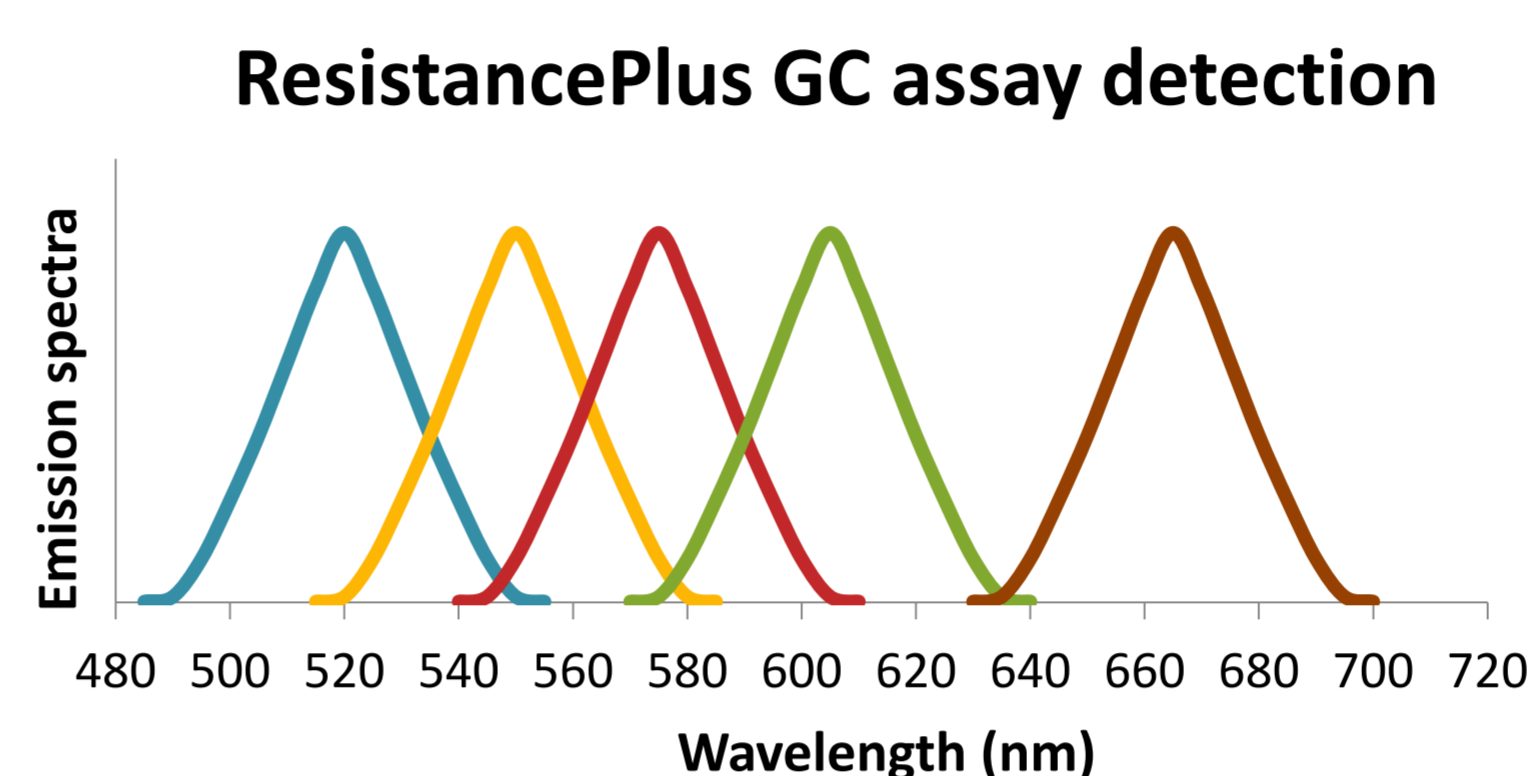
Methods

168 known GC PCR positive samples from a range of sites (Cervical/vaginal, Rectal, Urine or Throat) were anonymised and extracted using the automated nucleic acid extraction platform EASYMAG.

A ResistancePlus GC assay (SpeedX) was performed on these extracts and analyzed on ABI 7500 to detect for ciprofloxacin resistance in *N. gonorrhoeae*.

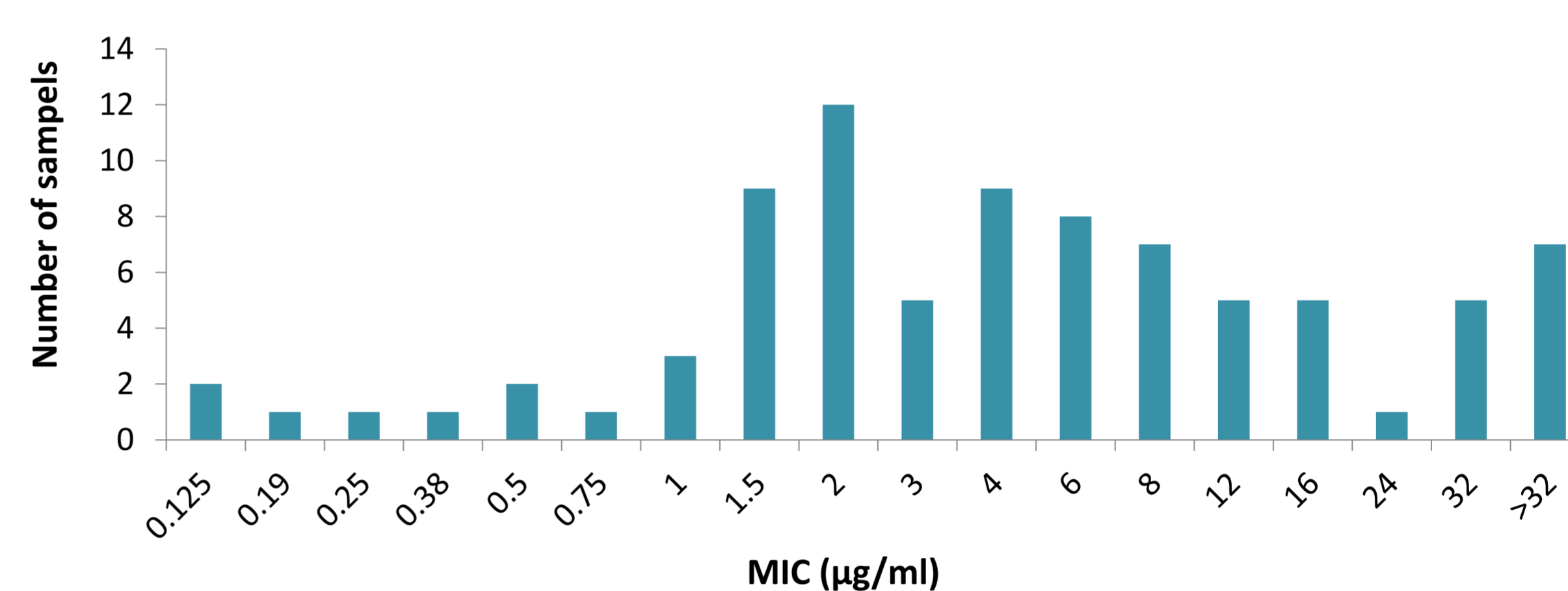
Antimicrobial susceptibility data was captured on all GC culture positives from 2015-2017 in Scotland by plate dilution and E-test.

Samples tested by the assay were compared to a corresponding culture isolate from the same site that was tested for ciprofloxacin susceptibility during the same GC episode (within 14 days).



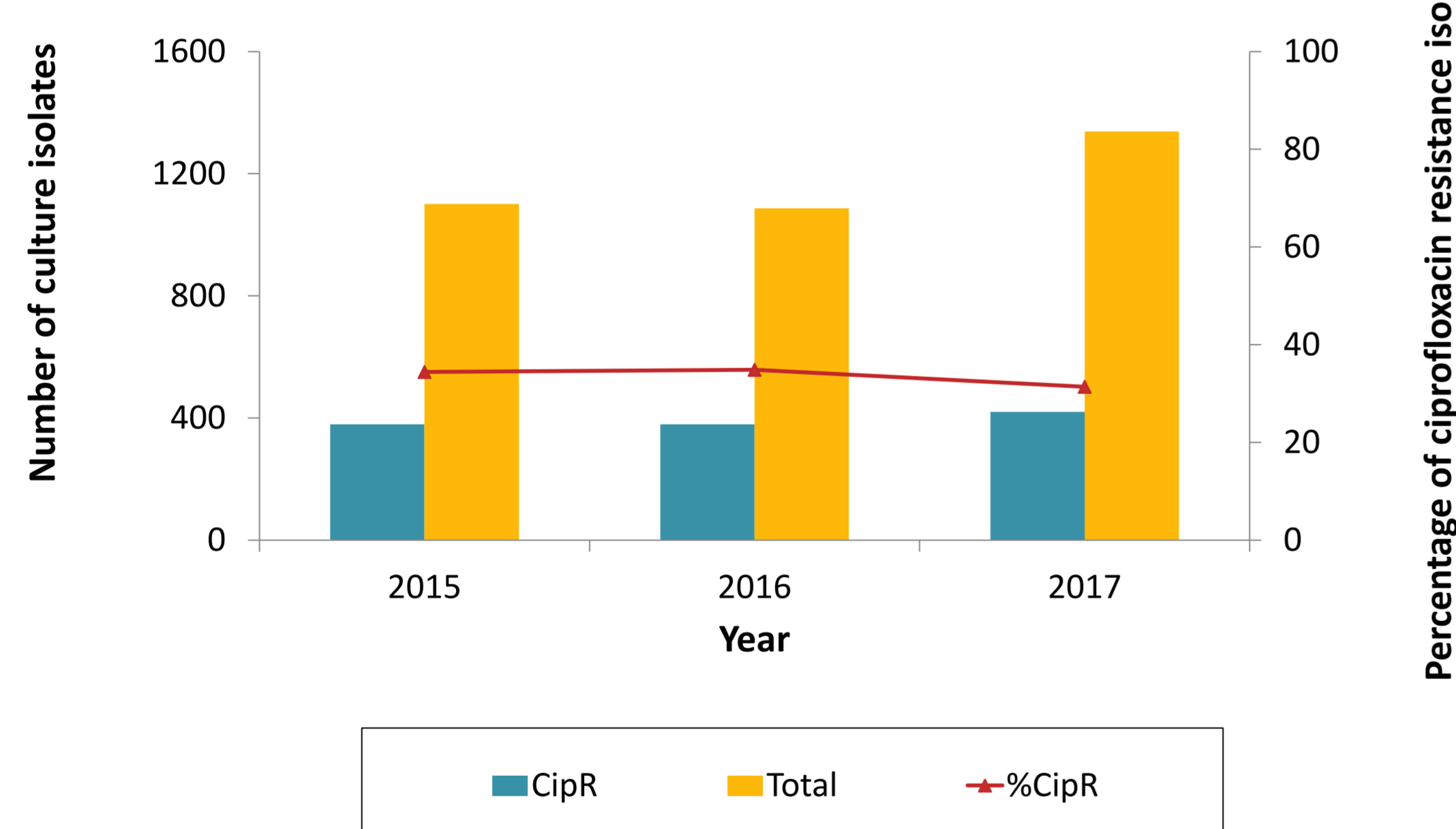
| Sample type | Corresponding culture isolates | | |
|------------------|--------------------------------|---|---|
| | Total | Sensitive to ciprofloxacin < 0.06 µg/ml | Resistant to ciprofloxacin > 0.06 µg/ml |
| Cervical/Vaginal | 47 | 24 | 23 |
| Rectal | 43 | 26 | 17 |
| Throat | 40 | 17 | 23 |
| Urine | 38 | 17 | 21 |
| Total | 168 | 84 | 84 |

MIC resistant ciprofloxacin culture isolates



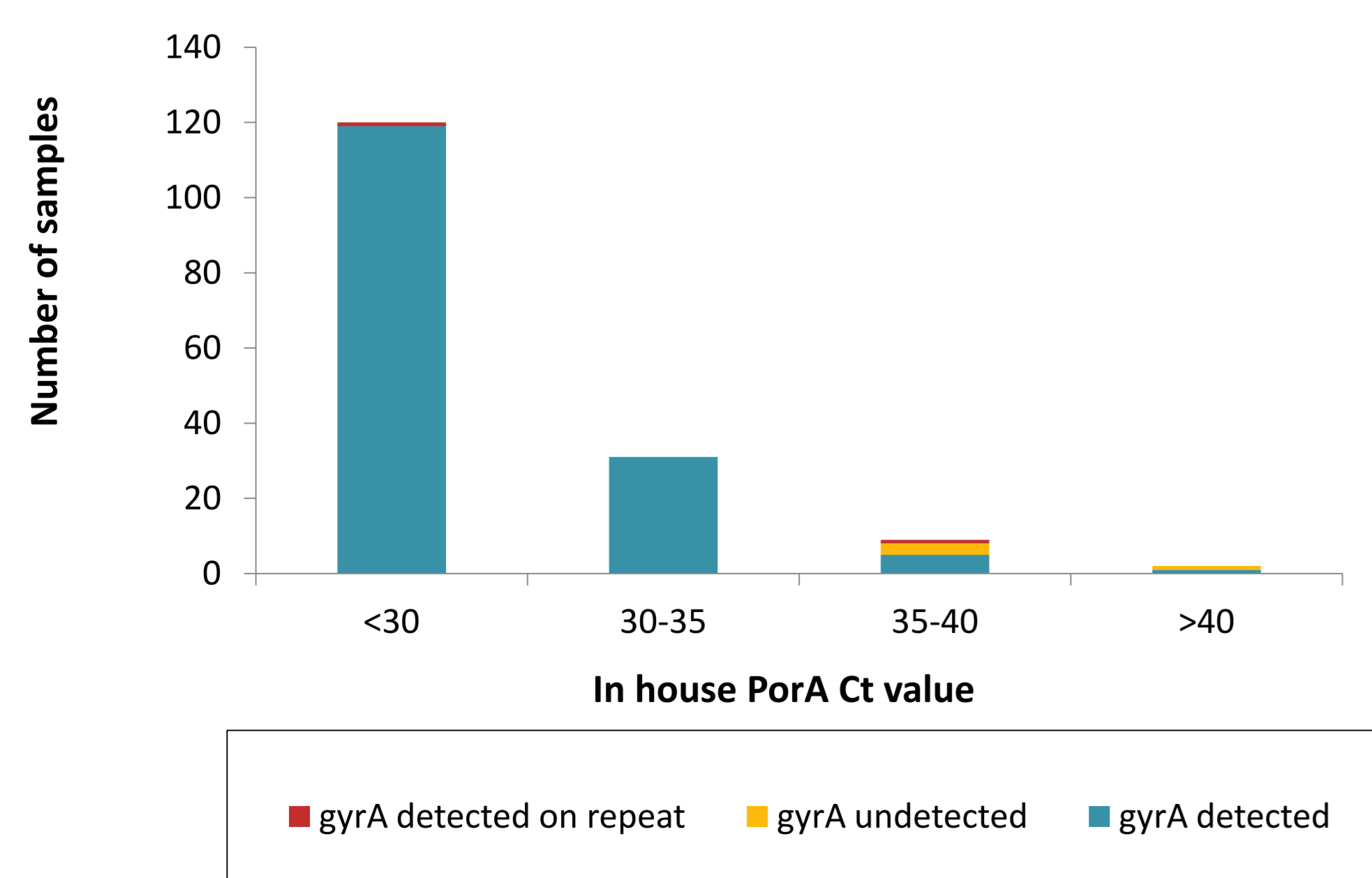
Results

GC Ciprofloxacin resistance



Ciprofloxacin resistance was seen in 379/1101 episodes in 2015 (34.4%), 379/1087 episodes in 2016 (34.8%) and 420/1338 episodes (31.4 %) in 2017.

GyrA detection less sensitive for weak positives



N. gonorrhoeae was detected in all of the samples (100 %) and *gyrA* in 164/168 samples (97.6 %).

4 samples with *gyrA* undetected had high Ct's (>35) in a 2nd line real-time PCR for *porA*. Ciprofloxacin resistance for these samples was indeterminate by the assay.

Assay agreement to culture phenotype



Disagree
Agree

| Sample type | Discrepant results | | |
|-------------|----------------------------------|----------------------------------|----------------------------------|
| | ResistancePlus GC PCR on extract | Ciprofloxacin E Test MIC (µg/ml) | ResistancePlus GC PCR on culture |
| Urine | WT | 4 | Mutant |
| Rectal | Mutant | 0.004 | WT |

In total 162/164 *gyrA* results matched the phenotype of a culture isolate taken from the same episode (98.7 %).

A ResistancePlus GC assay performed on the discrepant culture isolates matched the result of the culture phenotype and most likely the reason for discrepancy was due to mixed populations of susceptible and resistant *N. gonorrhoeae*.

The S91F mutation is very common in ciprofloxacin resistant isolates (>95%) as shown in this study and by others (Buckley *et al.* 2015 and Hemarajata *et al.* 2016).

Conclusions

The ResistancePlus GC assay performed well on clinical samples and could offer ciprofloxacin susceptibility testing within 4 hours to a laboratory service.

This would therefore allow for an alternative antibiotic to be prescribed and reduce the universal use of ceftriaxone.

Modeling based on current episodes in Scotland could mean that ciprofloxacin would be able to be used in >50 % of episodes which provides an exciting new approach to GC treatment.

References and Acknowledgements

Buckley. C., Trembizki. E., Donovan. B., Chen. M., Freeman. K. *et al.* 2015. A real-time PCR assay for direct characterization of the *Neisseria gonorrhoeae* GyrA 91 locus associated with ciprofloxacin susceptibility. *Journal of antimicrobial chemotherapy*. 71: 353-356.
Hemarajata. P., Yang. S., Soge. O., Humphries. R. and Klausner. J. 2016. Performance and Verification of a Real-Time PCR Assay Targeting the *gyrA* Gene for Prediction of Ciprofloxacin Resistance in *Neisseria gonorrhoeae*. *Journal of clinical microbiology*. 54: 805-808.
Shepherd. J., Wallace. L., Cullen. B., Goldberg. D. And Templeton. K. 2018. Gonococcal antibiotic surveillance in Scotland (GASS): prevalence, patterns and trends in 2017.

