Genomic analysis to understand non-typhoidal *Salmonella* carriage: *Salmonella* Agona – the bug that won't go away

[1&2] Winnie Lee, [2] Emma Waters, [3] Amina Ismail, [2] Gemma Langridge, [3] Marie Chattaway

[1] School of Cellular and Molecular Medicine, University of Bristol, Bristol, BS8 1TD, UK
[2] Quadram Institute Bioscience, Norwich Research Park, Norwich, NR4 7UQ
[3]Gastrointestinal Bacteria Reference Unit, United Kingdom Health Security Agency, London, England, NW9 5EQ

BACKGROUND

- *Salmonella* Agona is a non-typhoidal *Salmonella* known for biofilm production and persistence in the environment and dry food products[1,2]
- *S.* Agona has been associated with multi-country outbreaks, with 147 outbreak cases reported across 5 EU countries between 2014 and 2016[3] and is associated with shedding in pigs, sheep and gulls
- Persistent human infection can occur with *S.* Agona but the genetic basis for this versus sporadic infection is unknown
- Alongside traditional measures of variation (e.g. SNPs), many *Salmonella* serovars, including *S.* Agona, display chromosomal rearrangements that occur by recombination between the seven chromosomal copies of rRNA (*rrn)* operons[4] which may play a role in infection and/or persistence

AIMS

- Identify isolates responsible for a range of *S.* Agona infection in humans, from sporadic infection to chronic carriage (persistent)
- Perform comparative genomics using a combination of Nanopore and Illumina whole genome sequencing

METHODS

- Illumina sequencing data for 1,155 *S.* Agona isolates from infections in England were provided by UKHSA. A subset of 208 isolates were selected for high molecular weight DNA extraction using the Fire Monkey kit (RevoluGen) and Oxford Nanopore MinION long-read sequencing using a 48-plex, native ligation method
- MinION data was basecalled and demultiplexed with guppy. Long-read assemblies were generated using Flye, and polished with two rounds of Racon and one round of Medaka using long read data and corresponding overlapped reads generated by Minimap2. Hybrid assemblies were then generated by further polishing the final long read assembly with two rounds of Pilon using short read data and corresponding overlapped reads generated by Minimap2. Socru[5] was used to determine the order and orientation of the *S.* Agona genomes
- Maximum likelihood trees were constructed using RaxML. Snippy was utilised for core genome alignment. Gubbins was used to mask recombinant sequences in the alignment

RESULTS

1. Isolates associated with carriage are distributed across the phylogeny and intermix with isolates that cause acute infection

Figure 1. Maximum likelihood tree representing the core *S.* Agona genomes displaying the carriage status: sporadic, green; same episode blue; carriage, brown

2. Whilst the majority of *S.* Agona isolates had the conserved arrangement of GS1.0, 7 imbalanced rearrangements were identified

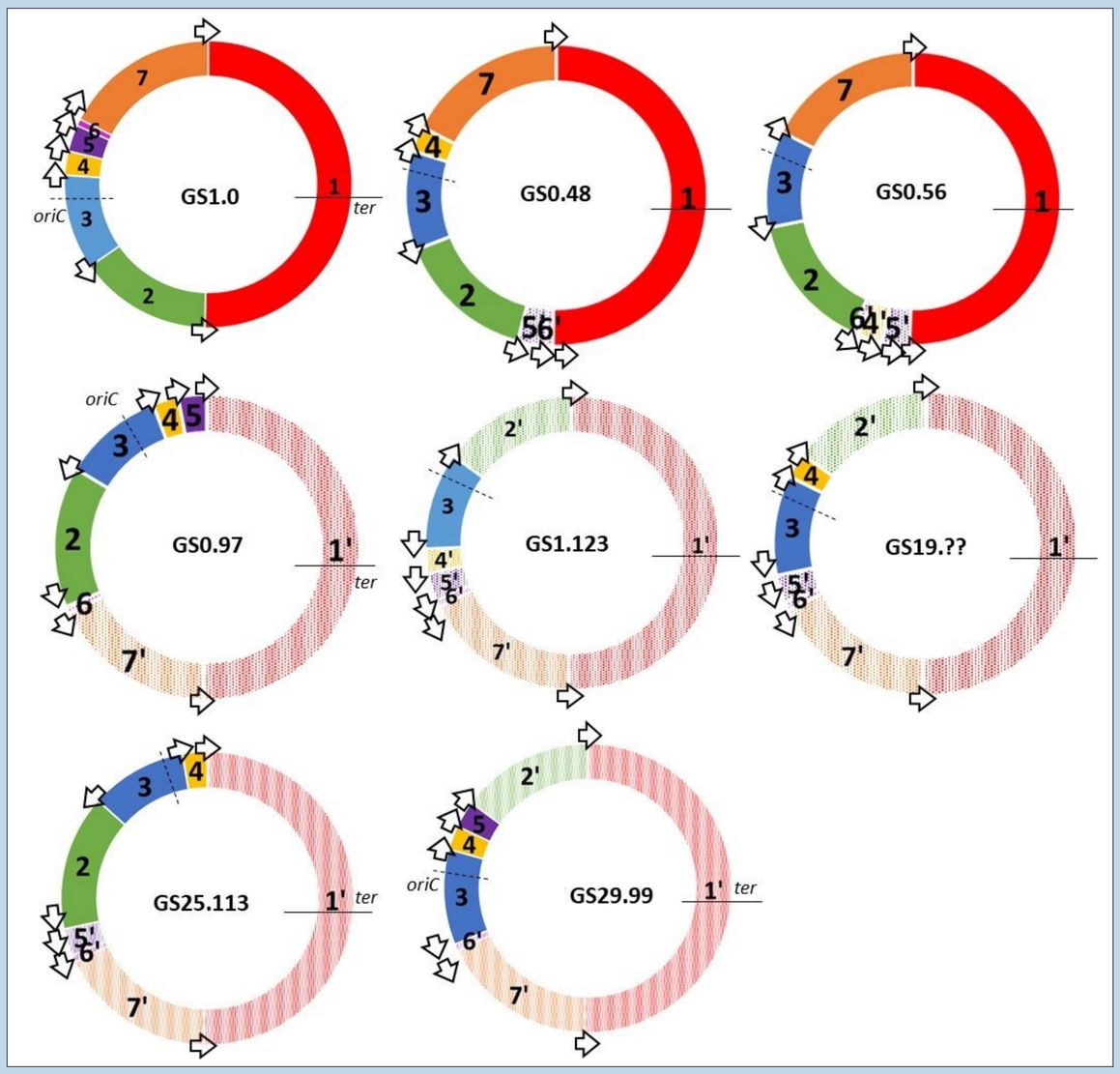

Figure 2. A comparison of GS1.0 and 7 other imbalanced genome structures in 11 different isolates, where the origin and terminus were closer together than in GS1.0, producing one shorter and one longer replichore

3. New genome structures have genotypic resistance to at least two classes and are mostly associated with carriage, with 3 structures that have acute infection status

Sample No.	SRR No.	Organism	GS Structure	Specimen	Year of isolation	Carriage status	Country	Resistance genes
41	SRR13833655	*S.* Agona	GS0.56 15'4'6'237	Human	2012	Acute	England	fosA7 aac(6')-Iaa
51	SRR5584683	*S.* Agona	GS 0.97 1'7'6'2345	Human	2012	Acute	England	fosA7 aac(6')-Iaa
52	SRR1645560	*S.* Agona	GS1.123 1'7'6'5'4'32'	Human	2013	Acute	England	tet(A) sul1 dfrA5 aac(6')-Iaa fosA7
87	SRR15460232	*S.* Agona	GS25.113 1'7'6'5'234	Human	2008	Carriage	England	aac(6')-Iaa parC fosA7
89	SRR1274899	*S.* Agona	GS1.123 1'7'6'5'4'32'	Human	2008	Carriage	Wales	fosA7 aac(6')-Iaa
90	SRR15299908	*S.* Agona	GS 0.97 1'7'6'2345	Human	2008	Carriage	England	fosA7 aac(6')-Iaa
93	SRR13785943	*S.* Agona	GS0.48 16'5'2347	Human	2008	Carriage	England	fosA7 aac(6')-Iaa
115	SRR1969818	*S.* Agona	GS19.?? 1'7'6'5'342'	Human	2015	Carriage	England	aadA7 sul1 tet(A) aac(6')-Iaa fosA7
138	SRR8671286	*S.* Agona	GS0.97 1'7'6'2345	Human	2016	Carriage	England	fosA7 aac(6')-Iaa
174	SRR7458804	*S.* Agona	GS29.99 1'7'6'3452'	Human	2018	Carriage	England	aac(6')-Iaa fosA7
178	SRR7184566	*S.* Agona	GS29.99 1'7'6'3452'	Human	2018	Carriage	England	aac(6')-Iaa fosA7

Figure 3. Metadata for the 11 *S.* Agona samples that have different genome structures to GS1.0 along with AMR genes

CONCLUSIONS

- This study reveals that human carriage of *S.* Agona occurs over a range of timeframes and several new genome structures have been identified, most of which are associated with carriage
- These findings emphasize the necessity for investigation of how genome rearrangements affect the behaviour of the bacterium

FUTURE DIRECTIONS

- Comparison of the levels and prevalence of AMR in this dataset to the public database of *S.* Agona
- With genome structures we have identified, we can begin a phenotypic investigation into whether the imbalanced genome structures play a role in their ability to form biofilms

ACKNOWLEDGEMENTS

I would like to thank the following:
- Supervisors: Gemma Langridge, Marie Chattaway and Emma Waters
- Langridge Lab at Quadram Institute, Norwich
- Revolugen
- Medical Research Foundation for giving me the opportunity to do a placement

REFERENCES

[1]Galié Serena, García-Gutiérrez Coral, Miguélez Elisa M., Villar Claudio J., Lombó Felipe. (2018). Biofilms in the Food Industry: Health Aspects and Control Methods. *Frontiers in Microbiology, 9*(898). DOI=10.3389/fmicb.2018.00898
[2] Díez-García, M., Capita, R. & Alonso-Calleja, C. (2012). Influence of serotype on the growth kinetics and the ability to form biofilms of Salmonella isolates from poultry. *Food Microbiology, 31*(2).
[3]European Centre for Disease Prevention and Control/European Food Safety Authority. Multi-country outbreak of Salmonella Agona possibly linked to ready-to-eat food – 26 July 2018. Stockholm and Parma: ECDC/EFSA; 2018 (https://www.ecdc.europa.eu/sites/default/files/documents/2018_07_ECDC-EFSA_ROA_UI-478_S_Agona_UK.pdf)
[4]Lui, S. & Sanderson, K. (1998). Homologous recombination between *rrn* operons rearranges the chromosome in host-specialised species of *Salmonella*. *FEMS Microbiology Letters, 164*(2), 275-281.
[5]Page, A., Ainsworth, E. V.& Langridge, G.C. *socru:* typing of genome-level order and orientation around ribosomal operons in bacteria. *Microbial Genomics, 6*(7). DOI= https://doi.org/10.1099/mgen.0.000396