Genomic analysis of ST88 Community-Acquired methicillin resistant *Staphylococcus aureus* in Ghana

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Background. The emergence and evolution of community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) strains in Africa is poorly understood. However, one particular MRSA lineage called ST88, appears to be rapidly establishing itself as an "African" CA-MRSA clone. In this study, we employed whole genome sequencing to provide more information on the genetic background of ST88 CA-MRSA isolates from Ghana and to describe in detail ST88 CA-MRSA isolates in comparison with other MRSA lineages worldwide. **Methods**. We first established a complete ST88 reference genome (AUS0325) using PacBio SMRT sequencing. We then used comparative genomics to assess relatedness among 17 ST88 CA-MRSA isolates recovered from patients attending Buruli ulcer treatment centers in Ghana, three non-African ST88s and 15 other MRSA lineages. **Results**. We show that Ghanaian ST88 forms a discrete MRSA lineage (harbouring SCC*mec*-IV [2B]). Gene content analysis identified five distinct genomic regions enriched among ST88 isolates compared with the other *S. aureus* lineages. The Ghanaian ST88 isolates had only 658 core genome SNPs and there was no correlation between phylogeny and geography, suggesting the recent spread of this clone. The lineage was also resistant to multiple classes of antibiotics including ß-lactams, tetracycline and chloramphenicol. **Discussion**. This study reveals that *S. aureus* ST88-IV is a recently emerging and rapidly spreading CA-MRSA clone in Ghana. The study highlights the capacity of small snapshot genomic studies to provide actionable public health information in resource limited settings. To our knowledge this is the first genomic assessment of the ST88 CA-MRSA

clone.

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Abstract

Background. The emergence and evolution of community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) strains in Africa is poorly understood. However, one particular MRSA lineage called ST88, appears to be rapidly establishing itself as an ``African´´ CA-MRSA clone. In this study, we employed whole genome sequencing to provide more information on the genetic background of ST88 CA-MRSA isolates from Ghana and to describe in detail ST88 CA-MRSA isolates in comparison with other MRSA lineages worldwide.

Methods. We first established a complete ST88 reference genome (AUS0325) using PacBio SMRT sequencing. We then used comparative genomics to assess relatedness among 17 ST88 CA-MRSA isolates recovered from patients attending Buruli ulcer treatment centers in Ghana, three non-African ST88s and 15 other MRSA lineages.

Results. We show that Ghanaian ST88 forms a discrete MRSA lineage (harbouring SCC*mec*-IV [2B]). Gene content analysis identified five distinct genomic regions enriched among ST88 isolates compared with the other *S. aureus* lineages. The Ghanaian ST88 isolates had only 658 core genome SNPs and there was no correlation between phylogeny and geography, suggesting the recent spread of this clone. The lineage was also resistant to multiple classes of antibiotics including ß-lactams, tetracycline and chloramphenicol.

41 **Discussion**. This study reveals that *S. aureus* ST88-IV is a recently emerging and rapidly spreading CA-
42 MRSA clone in Ghana. The study highlights the capacity of small snapshot genomic studies to provide
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Introduction

Since the 1990s, community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have been increasing worldwide (CDC, 2013; de Kraker, Davey & Grundmann, 2011). CA-MRSA clones are known to be more virulent than hospital-acquired MRSA, with infections linked to significant mortality and morbidity (Chambers, 2001; Chua et al., 2014; Chua et al., 2011; Etienne, 2005; Kourbatova et al., 2005; Seybold et al., 2006). First reported in Australia and the United States, CA-MRSA occurrence has been increasing, with epidemics due to clones such as ST8 USA300 in the United States (Diekema et al., 2014), ST93 and ST1 in Australia (Coombs et al., 2009), ST80 in Europe (Otter & French, 2010), ST59 in China and Taiwan (Chen & Huang, 2014), ST772 in India (D'Souza, Rodrigues & Mehta, 2010; DeLeo et al., 2010; Nadig et al., 2012; Shambat et al., 2012) and ST72 in South Korea (Kim et al., 2007). Other identified CA-MRSA clones belong to ST30 (South West Pacific clone) (Williamson, Coombs & Nimmo, 2014), ST45 (Berlin clone) (Witte et al., 1997), ST1 (USA400) (DeLeo et al., 2010) and ST78 (Western Australian MRSA-2) (Williamson, Coombs & Nimmo, 2014). In Africa, the distribution of MRSA clones in general is not well understood (Abdulgader et al., 2015). A recent review on MRSA in Africa with data from 15 of the 54 countries identified community clones of ST8-IV [2B] (USA300) and ST88-IV [2B] “West Australia MRSA-2 clone” in both community and health care associated infections in seven countries and a “Brazilian/Hungarian clone” ST239-III [3A] in hospital acquired infections in nine countries (Abdulgader et al., 2015). The European ST80-IV [2B] clone was limited to Algeria, Egypt and Tunisia while clonal types ST22-IV [2B], ST36-II [2A], and ST612-IV [2B] have only been reported so far in South Africa (Abdulgader et al., 2015). Among the two CA-MRSA clones, the ST8IV [2B] clone is an internationally disseminated clone recognized in every continent except Antarctica (David &

Daum, 2010). The ST88-IV [2B] CA-MRSA clone however is predominant in Sub-Saharan Africa (West, Central and East Africa) with reported rates of 24.2-83.3% of all MRSA isolates (Schaumburg et al., 2014). Studies from Angola (Conceicao et al., 2014), Cameroon (Breurec et al., 2011), Gabon (Ateba Ngoa et al., 2012; Schaumburg et al., 2011), Ghana (Amissah et al., 2015; Egyir et al., 2013; Egyir et al., 2014), Madagascar (Breurec et al., 2011), Niger (Breurec et al., 2011), Nigeria (Ghebremedhin et al., 2009; Raji et al., 2013; Shittu et al., 2012) and Senegal (Breurec et al., 2011) have identified it as a major circulating clone within both hospital and community settings. It was also detected in children from West Africa who underwent surgery in Switzerland but had been hospitalized in their home countries prior to surgical treatment (Blanc et al., 2007). Globally, this clone has been identified in China (Yu et al., 2008) and Japan (Maeda et al., 2012) in lower proportion (5.3-12.5%) than in Africa and sporadically in Belgium (Denis et al., 2005), Portugal (Aires-de-Sousa, Correia & de Lencastre, 2008) and Sweden (Fang et al., 2008).

Control of MRSA infections is assisted by a thorough knowledge of the epidemiology and dissemination of specific clones. To this end we employed whole genome sequencing and comparative genomics to describe in detail ST88 CA-MRSA isolates in comparison to other MRSA lineages worldwide.

Materials & Methods

Bacterial isolates and antibiogram analysis

The 17 ST88 *S. aureus* isolates analyzed from Ghana are listed in Table 1 and comprised five strains isolated in the Akwapim South District (Eastern Region) of Ghana with previously published genome data (GenBank accession numbers LFNJ00000000, LFNI00000000, LFNH00000000, LFMH00000000, LFMG00000000) (Amissah et al., 2015) and 12 isolates recovered from wounds of 11 patients attending Buruli ulcer (BU) treatment centers in the Ga-South and Ga-West municipalities (Greater Accra Region) of Ghana with two isolates from one patient; one a PVL positive isolate and the other PVL negative. Patients were outpatients, nine of whom had laboratory confirmed BU. Initial isolate identification was made using colony and microscopic morphology, catalase and coagulase biochemical reactions and a Staphylase kit BD BBL™ Staphyloslide Latex Test (Becton, Dickinson and Company) for further confirmation. Antibiograms were determined using the Kirby Bauer disc diffusion method according to CLSI guidelines (CLSI, 2014) and PCR targeting the *mecA* gene (Oliveira & de Lencastre, 2002) for identification of MRSA. Ethical clearance was obtained from the institutional review board of the Noguchi Memorial Institute for Medical Research (NMIMR) (Federal-wide Assurance number FWA00001824). All study participants were well informed of the study objectives and written informed consent was obtained either from the patient or from the guardian of the patient.

DNA Extraction, Whole Genome Sequencing and Analysis

Genomic DNA was extracted from isolates using the Qiagen DNeasy kit and protocol (Qiagen, Hilden, Germany). DNA libraries were prepared using Nextera XT (Illumina, San Diego, California, USA) and whole genome sequencing was performed using the Illumina MiSeq with 2 x 300 bp chemistry. Small Molecular Real Time sequencing was performed on the RS-II (Pacific

Biosciences) using P6-C4 chemistry, and reference genome assembly was performed as described (Baines et al., 2016).

Read mapping, variant calling and phylogenomic analysis

The sequence reads were processed using *Nullarbor* (nullarbor.pl 0.6, https://github.com/tseemann/nullarbor), a recently developed bioinformatics pipeline for public health microbial genomics as described previously (Kwong et al., 2016). *S. aureus* ST88 raw sequence reads with accession numbers ERS1354589-600 have been deposited in the European Nucleotide Archive (ENA), Project PRJEB15428 (url - http://www.ebi.ac.uk/ena/data/view/PRJEB15428. Ortholog clustering was performed using Roary (Page et al., 2015) and was visualized with Fripan (http://drpowell.github.io/FriPan/). Recombination within the core genome was inferred using ClonalFrameML v1.7 (Didelot & Wilson, 2015) with the whole genome alignment generated by Nullarbor. Using FastTree v2.1.8 (Price, Dehal & Arkin, 2010), a ML tree was generated and used as a guide tree for ClonalFrameML. Positions in the reference genome that were not present in at least one genome (non-core) were omitted from the analysis using the "ignore_incomplete_sites true" option and providing ClonalFrameML with a list of all non-core positions. Maximum likelihood trees were constructed using a recombination free SNP alignment using FastTree. Bootstrap support was derived from comparisons between the original tree against 1000 trees that were built upon pseudo-alignments (sampled from the original alignment with replacement).

Results and Discussion

136 **ST88 complete reference genome**

137 A prerequisite for high-resolution comparative genomics by read-mapping is a high-quality,
138 complete reference genome, closely related to the bacterial population under investigation
139 (Kwong et al., 2016). There were no fully-assembled ST88 *S. aureus* genomes publicly
140 available, so to address this issue, we selected the methicillin-susceptible, penicillin-resistant
141 ST88 *S. aureus* isolate AUS0325. This clinical isolate was obtained in 2013 from a patient in
142 Melbourne, Australia who had a persistent infection of a prosthetic joint, and was part of a
143 separate, unpublished study. The AUS0325 genome comprised a 2,771,577 bp circular
144 chromosome with 32.9% GC content. There were no plasmids; the beta-lactamase operon (*bla*)
145 was carried by the Tn*552* transposon and integrated into the chromosome. The overall
146 chromosome architecture of AUS0325 was like representative *S. aureus* genomes from other
147 community-associated lineages (ST1, ST8 and ST93) but with five distinct regions of difference,
148 discussed in more detail below (Fig. 1A). We took advantage of the PacBio data to define the
149 Sa_aus0325 methylome. Motif analysis and inspection of the AUS0325 annotation identified two
150 active type I restriction modification *hsdMS* loci. Protein alignment of the two *hsdS* alleles with
151 previously characterised *hsdS* proteins allowed the attribution of target recognition sequences to
152 either allele (Monk et al., 2015) (Table 2). The first *hsdS* recognized a motif not previously
153 described, while the second *hsdS* contained an identical sequence to the target recognition
154 domain-2 of CC30-2 and ST93-2, which recognises TCG (Table 2).

155

156 **ST88 population structure**

To understand the genomic diversity and evolutionary origin of the ST88 isolates, a core genome phylogeny was inferred by mapping reads from the 17 ST88 isolates (Table 1, Fig. 1B), two published ST88 MRSA genomes from Lebanon and USA and 15 other geographically and genetically distinct *S. aureus* clones to AUS0325 (Table 3, Fig. 1B). To assess the clonal ancestry, SNPs within inferred regions of recombination (71,862 clonal SNPs; 26,570 recombinogenic SNPs) (Fig. S1) were removed and a maximum likelihood phylogenomic tree was established using the clonal core SNP alignment (71,862 SNPs). All 20 ST88 genomes formed a discrete, closely related lineage, defined by only 1,759 core genome SNPs, compared with 71,862 SNPs among all 35 *S. aureus* genomes (Fig. 1B, Fig. 2A). The global tree was rooted using *Bacillus_subtilis*_B4068 (GenBank ID: JXHK00000000) (Berendsen et al., 2016) as an outgroup and this phylogeny indicated ST88 shares a most recent common ancestor (MRCA) with ST72 (Fig. 1A).

Five distinct genomic regions were identified by ortholog comparisons, enriched among the ST88 genomes compared to the 15 other diverse *S. aureus* genomes. These regions included νSAα (GI-3, Fig. 1) that harboured 10 staphylococcal superantigen-like (*ssl*) genes, of which four were uniquely present in the ST88 isolates. Upregulation of SSLs has been reported in some CA-MRSA strains and may be involved in neutrophil and complement activation (Foster, 2005; Voyich et al., 2005). GI-3 also harboured the first of the two functional type I restriction modification *hsdMS* loci (see above, Table 2). GI-1 and GI-4 may be mobile integrative elements of unknown function with the presence of putative integrases and four and 12 CDS respectively, all encoding hypothetical proteins. GI-1 also harbours elements of a putative restriction modification system (Fig. 1, Table S1). GI-2 contains 13 CDS, most of unknown function although three CDS may encode membrane proteins (Fig. 1, Table S1). GI-5 had 14 CDS, that

included the second of the type I restriction modification *hsdMS* loci and seven CDS encoding putative proteases (Table 2, Table S1).

Evolution and molecular epidemiology of ST88 in Ghana

To assess the evolutionary relationships among the ST88 genomes, a phylogenomic tree comprised exclusively of ST88 genomes was established using clonal, core SNPs (1,759 clonal SNPs; 207 recombinogenic SNPs) (Fig. S2, Fig. 2A). The tree was rooted using an ST93 genome (Sa_JKD6159) as an outgroup. The phylogeny and the restricted genomic diversity (658 core SNPs) suggests that the spread of ST88 MRSA in Ghana is a recent phenomenon, with isolates from the United States, Australia and Lebanon ancestral to the spread of these isolates in Ghana. Five specific clusters of CDS were also found to be exclusively present with the African ST88 genomes (Fig. 2C). These CDS were different to the five genomic regions identified in all ST88 relative to other *S. aureus* clones (Fig. 1B) suggesting that they were horizontally acquired by an ST88 MRCA that has since spread in Ghana, (although a significantly larger sampling effort would be required to confirm this hypothesis). These regions harbour CDS suggestive of plasmid, phage and other mobile DNA elements (Table S2). We conducted a phylogeographic analysis to formally assess the relationship between the Ghanaian ST88 phylogeny and the specific geographic origin of the isolates, based on patient villages. However, there was no correlation between geography and phylogeny, suggesting again that the spread of ST88 in Ghana has been recent and rapid (Fig. 3).

202 **Phenotypic and genotypic antibiotic resistance**

203 All 17 Ghanaian ST88 isolates harboured a SCC*mec*-IV [2B] cassette, and displayed phenotypic
204 resistance to β-lactams, tetracycline, and chloramphenicol (Table 1). Isolates were variably
205 resistant to erythromycin, clindamycin, trimethoprim, amikacin and streptomycin (Table 1).
206 There was agreement between phenotypic and inferred genotypic resistance (Fig. 1B). For the
207 four genes (*blaZ, mecA, tetL, tetM*) detected in all 12 ST88 isolates from the Greater Accra
208 Region, resistance correlated with phenotypic resistance to all β-lactams and tetracyclines. Six
209 isolates showed phenotypic and genotypic resistance to chloramphenicol (Table 1, Fig. 1B). Five
210 of these isolates were from the same health center, however the time of isolation and the
211 geographic origins of the patients were different, suggesting that these isolates are spread across
212 the region and were not acquired from a common source.

213

214 **Conclusions**

215 The analysis presented here suggests that *S. aureus* ST88-IV is an emerging CA-MRSA clone in
216 Ghana. This has the potential to become a serious public health threat, with implications for the
217 treatment of *S. aureus* infections in Ghana, where there is no developed surveillance
218 infrastructure to monitor antibiotic resistance. The abuse and misuse of antibiotics by health care
219 givers and patients in Ghana is extensive (Kpeli et al., 2016). The development of resistance is
220 furthermore encouraged by the widespread availability of higher classes of antibiotics to lower
221 level health centers from regional medical stores, in addition to the unrestricted sale of these
222 medicines to over-the-counter medicine sellers by pharmaceutical wholesalers- even though
223 existing laws are supposed to limit the scope of these facilities to handle such medicines. Also

implicated and widely documented are the prescribing practices of clinicians; with over-reliance on presumptive treatment and haphazardly prescribing antibiotics without recourse to due laboratory investigation. CA-MRSA has undergone rapid evolution and expansion worldwide. Because of its epidemic potential and limited treatment options, vigilance and antibiotic stewardship programmes need to be put in place to prevent further spread.

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Table 1(on next page)

S. aureus ST88 isolates tested in this study

1 **Table 1**: *S. aureus* ST88 isolates tested in this study

Isolate ID	Origin (Ghana)	Phenotypic resistance*	Genotype (*spa*, *agr*, PVL)	Reference
Sa_NOG-W02	Greater Accra Region	cld, tet, amp, ery, fox, ctx, chl, cro	t939, agr-3, PVL +	This study
Sa_NOG-W25	Greater Accra Region	gen, amk, cld, str, amp, tet, sxt, cfx, ctx, chl, cro	t448, agr-3, PVL -	This study
Sa_NOG-W11	Greater Accra Region	str, amk, gen, sxt, cfx, cld, fox, ctx, tet, chl, cro, amp, ery	t186, agr-3, PVL +	This study
Sa_NOG-W13	Greater Accra Region	gen, str, amk, ctx, tet, chl, cro, sxt, cfx, amp, cld, fox	07-12-12-118-13-13, agr-3, PVL +	This study
Sa_NOG-W01	Greater Accra Region	amk, cfx, tet, ctx, chl, cro, fox	t186, agr-3, PVL +	This study
Sa_NOG-W10	Greater Accra Region	sxt, ery, gen, str, amk, cld, amp, cfx, tet, fox, ctx, chl, cro	t186, agr-3, PVL -	This study
Sa_NOG-W07	Greater Accra Region	gen, str, amp, tet, sxt, cfx, chl, cro, ctx, fox, cld, ery,	t448, agr-3, PVL -	This study
Sa_NOG-W14	Greater Accra Region	gen, ery, sxt, amk, cld, str, tet, amp, cfx, ctx, chl, cro, fox,	t2649, agr-3, PVL +	This study
Sa_NOG-W04	Greater Accra Region	sxt, ery, gen, str, amk, amp, cfx, tet, fox, ctx,	07-12-21-17-13-13-34-34-33-34-34, agr-3,	This study

		chl, cro	PVL -	
Sa_NOG-W06	Greater Accra Region	sxt, gen, amk, cld, amp, tet, cfx, fox, chl, cro	t786, agr-3, PVL -	This study
Sa_NOG-W24	Greater Accra Region	gen, sxt , amk, str, amp, tet, cfx, ctx, chl, cro, fox,	t786, agr-3, PVL +	This study
Sa_NOG-W05	Greater Accra Region	ery, amk, str, amp, cfx, tet, sxt, cld,	t186, agr-3, PVL -	This study
BU_G0701_t5	Eastern Region	fox, ben, oxa, tet, chl	t786, agr-3, PVL -	(Amissah et al., 2015b)
BU_G0201_t8	Eastern Region	fox, ben, oxa, tet, chl	t786, agr-3, PVL -	(Amissah et al., 2015b)
BU_G0202_t2	Eastern Region	fox, ben, oxa, tet, chl	t786, agr-3, PVL -	(Amissah et al., 2015b)
BU_G1905_t3	Eastern Region	fox, ben, oxa, tet, chl	t786, agr-3, PVL -	(Amissah et al., 2015b)
BU_W13_11	Eastern Region	fox, ben, oxa, tet, chl	t186, agr-3, PVL -	(Amissah et al., 2015b)

Notes: *oxacillin=oxa, cefoxitin=fox, tetracycline=tet, chloramphenicol=chl, cefuroxime=cfx, erythromycin=ery, clindamycin=cld, sulphamethxazole-trimethoprim=sxt, amikacin=amk, streptomycin=str, ampicillin=amp, cefotaxime=ctx, ceftriaxone=cro, gentamicin=gen, benzylpenicillin=ben, *spa*=*Staphylococcus aureus* Protein A, *agr*=Accesory Gene regulator, PVL=Pantone Valentine Leukocidin toxin

9

Table 2(on next page)

Sa_aus0325 methylome analysis

Table 2: Sa_aus0325 methylome analysis

HsdS (nucleotide position)	TRD1	N	TRD2
397,724 -> 399,280	ACC	5	RTGT
1,849,852 <- 1,851,408	GAG	6	TCG

Table 3(on next page)

Comparator Reference Genomes

1 **Table 3:** Comparator Reference Genomes

Sequence type	Region/Country of origin	MSSA/MRSA	Reference Strain	Assembly/Accesssion number
ST8	USA/Canada	CA-MRSA	*Staphylococcus aureus* subsp. *aureus* USA 300 FPR 3757	NC_007793.1
ST 1	USA/Canada	CA-MRSA	*Staphylococcus aureus* subsp. *aureus* MW2	NC_003923.1
ST 80	Europe	CA-MRSA	*Staphylococcus aureus* 11819-97	NC_017351.1
ST45	Europe/ USA/Canada	CA-MRSA	*Staphylococcus aureus* subsp. *aureus* 300-169	GCA_000534855.1
ST 30	Europe/ Australia/Asia	CA-MRSA	*Staphylococcus aureus* subsp. *aureus*_S2397	GCA_000577595.1
ST 72	Asia	CA-MRSA	*Staphylococcus aureus* MRSA_CVM43477	GCA_000830555.1
ST 59	Asia	CA-MRSA	*Staphylococcus aureus* subsp. *aureus* M013	NC_016928.1
ST93	Australia	CA-MRSA	*Staphylococcus aureus* subsp. *aureus* JKD 6159	NC_017338.1
ST 250	England	HA-MRSA	*Staphylococcus aureus* subsp. *aureus* COL	NC_002951.2
ST254	Japan	MSSA	*Staphylococcus aureus* subsp. *aureus* Newman	NC_009641.1
ST1	United Kingdom	MSSA	*Staphylococcus aureus* subsp. *aureus* MSSA476	NC_002953.3
ST5	Ireland	MSSA	*Staphylococcus aureus* subsp. *aureus* ED98	NC_013450.1
ST5	Japan	MRSA	*Staphylococcus aureus* subsp. *aureus* N315	NC_002745.2
ST 239	Australia	MRSA	*Staphylococcus aureus* subsp. *aureus* JKD 6008	NC_017341.1

ST772	India	MRSA	*Staphylococcus aureus* subsp. *aureus*_ST772-MRSA	GC_000516935.1
ST 88	Lebanon	MRSA	HST-105	GCA_000564895.1
ST 88	United States	MSSA	*Staphylococcus aureus* subsp *aureus*_21343	GCA_000245595.2

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Figure 1

Comparative genomic analysis of *S. aureus* ST88

(A) DNA-DNA comparisons visualized using the Artemis Comparison Tool of three CA-MRSA representative chromosomes and S. aureus COL against the complete chromosome of ST88 isolate AUS0325. (B) Core genome phylogeny and accessory genome elements identified among ST88 isolates. The phylogeny was based on an alignment of 71,862 non-recombinogenic core genome SNPs (indels excluded) and inferred using FastTree. Nodes with greater than 70% bootstrap support (1000 replicates) are labelled with red dots. Antibiotic resistance genes were identified using Abricate (https://github.com/tseemann/abricate) and genomic islands (GIs) enriched among ST88 isolates were identified by ortholog comparisons using Roary and visualized using FriPan. CDS present in specific GI are listed in Table S1.

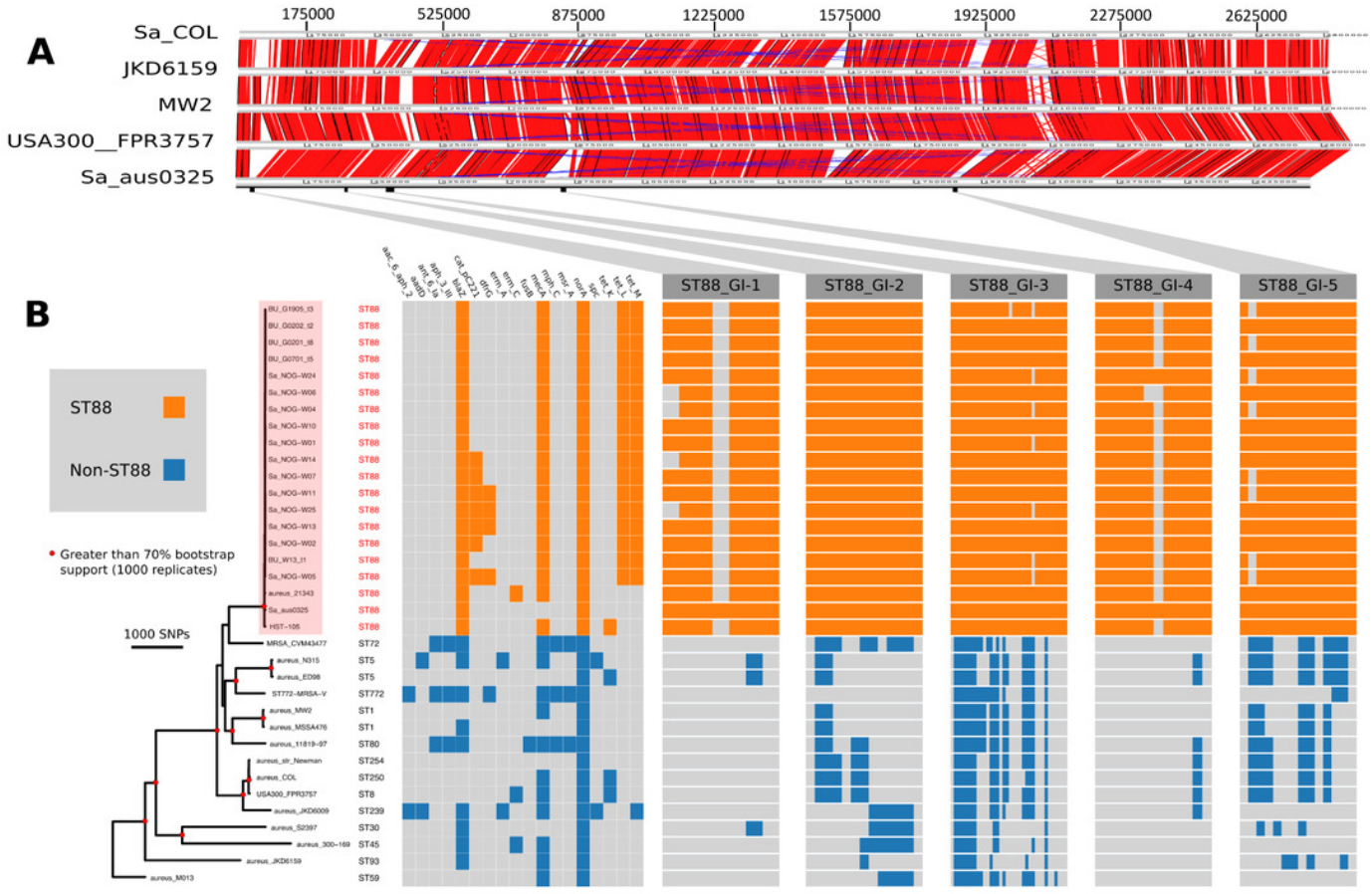


Figure 2

High resolution ST88 phylogeny and accessory genome analysis

(A). Phylogeny inferred by read-mapping and variant identification among only ST88 genomes. Tree was produced using FastTree based on a pairwise alignment of 1,759 non-recombinogenic core genome SNPs among the 20 ST88 genomes. All major nodes in the tree (red circles) had greater than 70% bootstrap support (1000 replicates). (B) Accessory gene content variation among the 20 ST88 genomes as assessed by ortholog comparisons using Roary. (C) Distinct genomic islands (GI) identified in Ghanaian isolates.

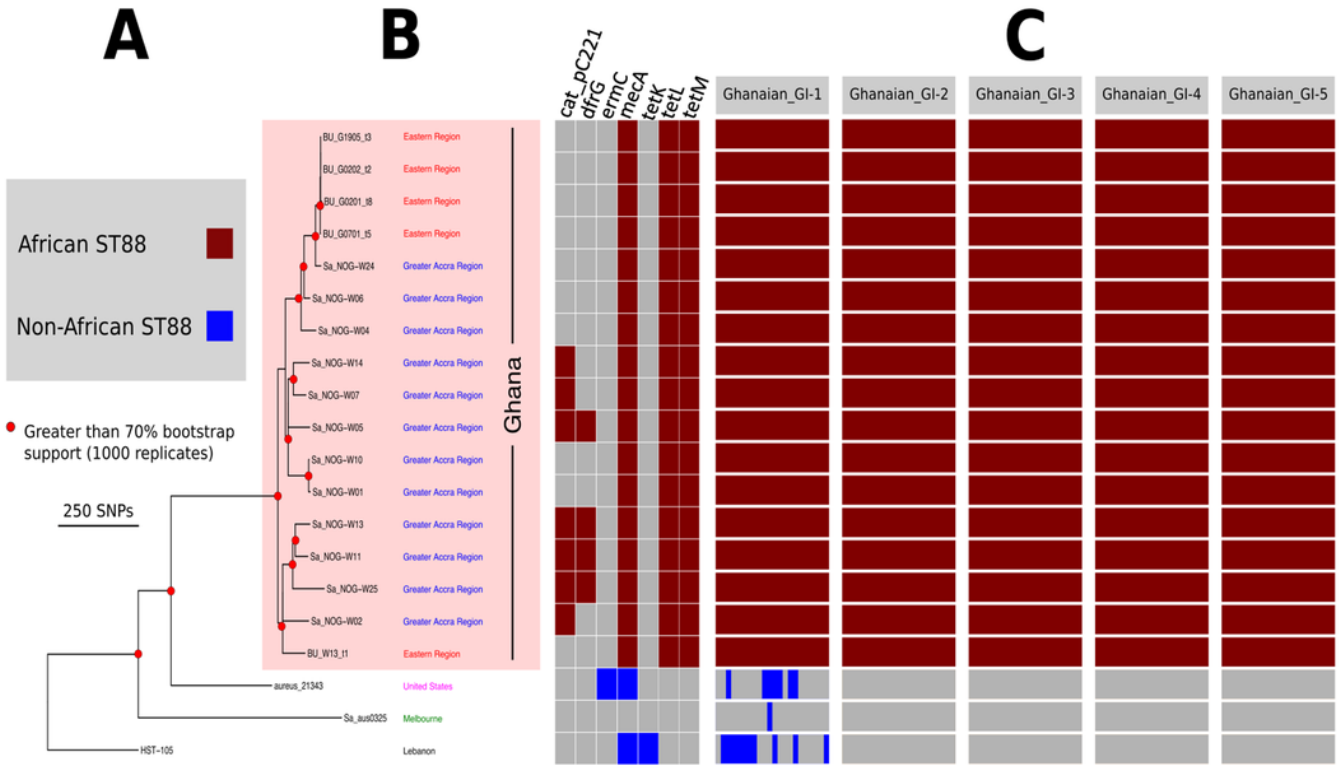
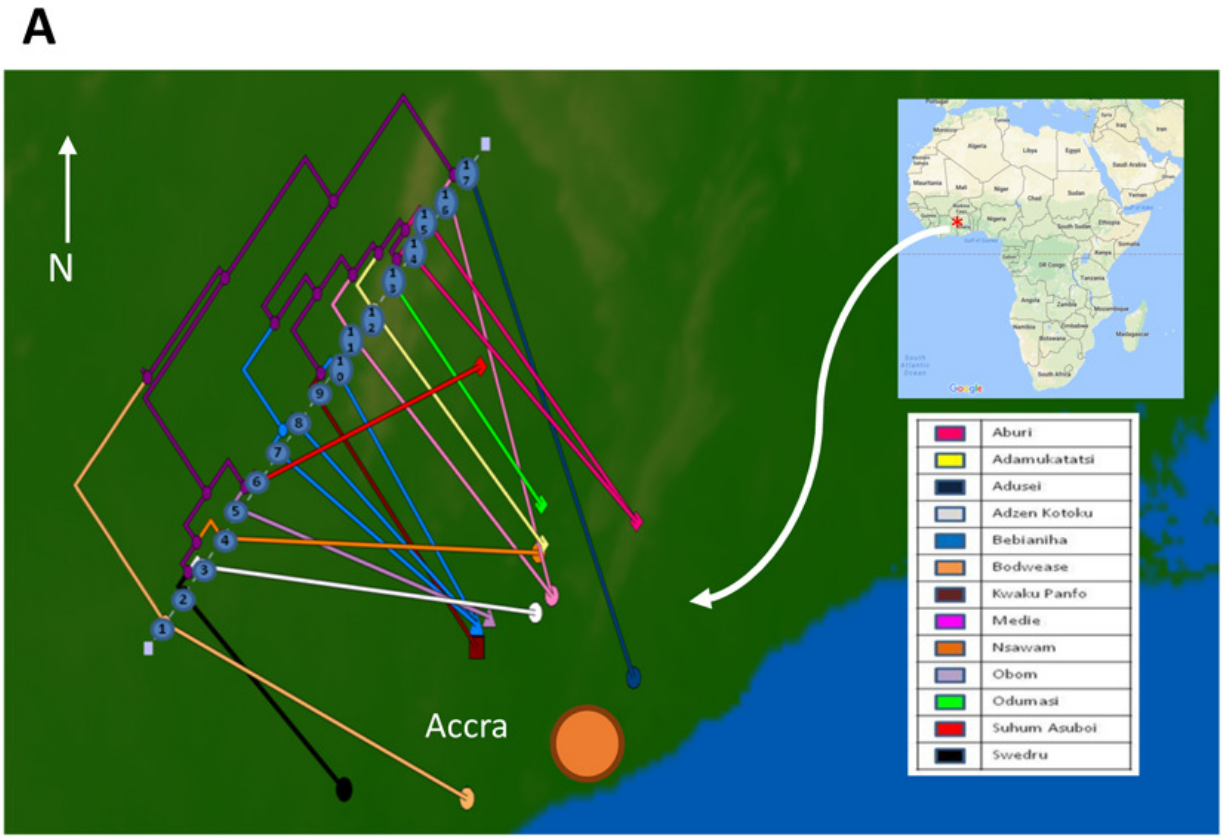
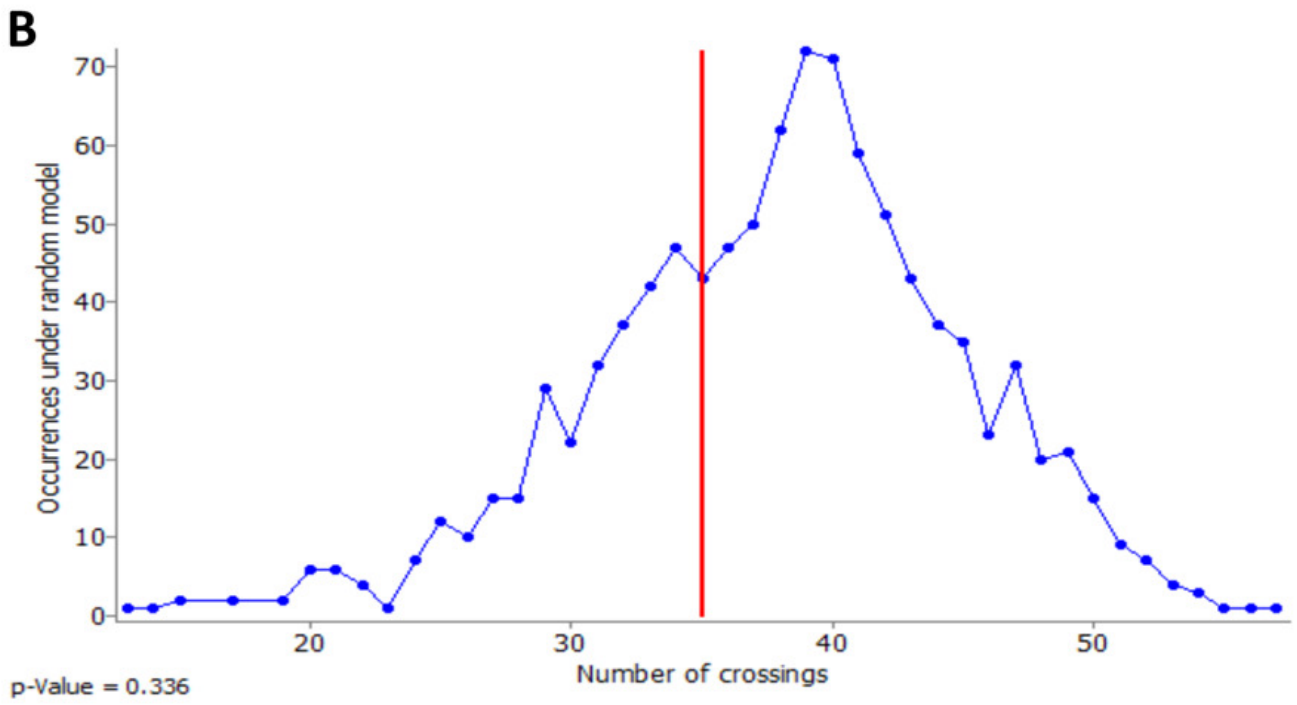


Figure 3

Relationship between phylogeny of Ghanaian ST88 and their geographic origin

(A) Phylogeographic alignment of phylogeny against isolate origin geography performed with GenGIS software and (B) Monte-Carlo analysis following 1000 permutations of tree tips and geography of originating villages. The arrangement derived from the data was not significantly different to that which is expected by chance alone (p value >0.05), indicating a lack of geographical structure among the ST88 genomes.


A
N
Accra
Aburi
Adamukatatsi
Adusei
Adzen Kotoku
Bebianiha
Bodwease
Kwaku Panfo
Medie
Nsawam
Obom
Odumasi
Suhum Asuboi
Swedru


B
Occurrences under random model
Number of crossings
p-Value = 0.336