

# A Novel Integrative Conjugative Element Mediates Genetic Transfer from Group G Streptococcus to Other $\beta$ -Hemolytic Streptococci<sup>∇</sup>

Mark R. Davies,<sup>1,2,3†</sup> Josephine Shera,<sup>1</sup> Gary H. Van Domselaar,<sup>4,5</sup>  
 Kadaba S. Sriprakash,<sup>1,2,3,6</sup> and David J. McMillan<sup>1,2,6\*</sup>

Bacterial Pathogenesis Laboratory, The Queensland Institute of Medical Research, Brisbane 4029, Queensland, Australia<sup>1</sup>; The Australian Centre for International Tropical Health and Nutrition, The Queensland Institute of Medical Research, Brisbane 4029, Queensland, Australia<sup>2</sup>; Cooperative Research Centre for Vaccine Technology, The Queensland Institute of Medical Research, Brisbane 4029, Queensland, Australia<sup>3</sup>; National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba R3E 3R2, Canada<sup>4</sup>; Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada<sup>5</sup>; and Griffith Medical Research College, Herston, Queensland, Australia<sup>6</sup>

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**Lateral gene transfer is a significant contributor to the ongoing evolution of many bacterial pathogens, including  $\beta$ -hemolytic streptococci. Here we provide the first characterization of a novel integrative conjugative element (ICE), ICESde3396, from *Streptococcus dysgalactiae* subsp. *equisimilis* (group G streptococcus [GG]), a bacterium commonly found in the throat and skin of humans. ICESde3396 is 64 kb in size and encodes 66 putative open reading frames. ICESde3396 shares 38 open reading frames with a putative ICE from *Streptococcus agalactiae* (group B streptococcus [GBS]), ICESa2603. In addition to genes involved in conjugal processes, ICESde3396 also carries genes predicted to be involved in virulence and resistance to various metals. A major feature of ICESde3396 differentiating it from ICESa2603 is the presence of an 18-kb internal recombinogenic region containing four unique gene clusters, which appear to have been acquired from streptococcal and nonstreptococcal bacterial species. The four clusters include two cadmium resistance operons, an arsenic resistance operon, and genes with orthologues in a group A streptococcus (GAS) prophage. Streptococci that naturally harbor ICESde3396 have increased resistance to cadmium and arsenate, indicating the functionality of genes present in the 18-kb recombinogenic region. By marking ICESde3396 with a kanamycin resistance gene, we demonstrate that the ICE is transferable to other GGS isolates as well as GBS and GAS. To investigate the presence of the ICE in clinical streptococcal isolates, we screened 69 isolates (30 GGS, 19 GBS, and 20 GAS isolates) for the presence of three separate regions of ICESde3396. Eleven isolates possessed all three regions, suggesting they harbored ICESde3396-like elements. Another four isolates possessed ICESa2603-like elements. We propose that ICESde3396 is a mobile genetic element that is capable of acquiring DNA from multiple bacterial sources and is a vehicle for dissemination of this DNA through the wider  $\beta$ -hemolytic streptococcal population.**

Lateral gene transfer (LGT) plays a profound role in the generation of genetic diversity within bacterial pathogens (28, 35). LGT rapidly facilitates the adaptation of bacteria to novel environments and leads to the expansion of virulence determinants. Conjugative transposons are major mediators of LGT in prokaryotes and together with integrative plasmids are known as integrative conjugative elements (ICEs). ICEs often carry genes for auxiliary traits such as resistance to antibiotics and heavy metals and are widely implicated as the primary disseminator of such phenotypes (9).

The  $\beta$ -hemolytic streptococci constitute a group of human and animal pathogens that cause a wide variety of diseases in their respective hosts (24). The human pathogens include *Streptococcus pyogenes* (group A streptococcus [GAS]), *Streptococcus agalactiae* (group B streptococcus [GBS]), and *Streptococcus dysgalactiae* subsp. *equisimilis* (human group C and group G streptococcus [GG]). Historically, GAS is associated with diseases such as pharyngitis, impetigo, scarlet fever, post-streptococcal glomerulonephritis, rheumatic disease, and rheumatic heart disease (16). In the 1980s, GAS emerged as a cause of a serious and potentially fatal invasive disease (15, 47) which is estimated to kill between 1,000 and 1,700 people each year in the United States (6). Similarly, GBS, a major veterinary pathogen, emerged as a leading cause of bacterial invasive disease in newborns in the 1970s (21). GGS has traditionally been considered a commensal organism found as part of the normal flora of the skin, throat, and other mucosal surfaces and caused only opportunistic infections in individuals with underlying risk factors (12). However, GGS is increasingly associated with a spectrum of disease in healthy individuals which overlaps that of GAS. These include epidemic pharyngitis, bacteremia, puerperal sepsis, peritonitis, cellulitis, septicemia, infective endocarditis, and glomerulonephritis (12, 14, 22, 23, 29, 30, 34, 49, 56). Additionally, GGS is also associated with serious streptococcal invasive diseases, including necrotizing fasciitis and toxic shock syndrome (57).

Changes in genome content, arising through mutations (e.g., allelic variation or gene duplication) or LGT have been hy-

\* Corresponding author. Mailing address: Bacterial Pathogenesis Laboratory, Queensland Institute of Medical Research, Brisbane, Qld 4006, Australia. Phone: 61-7-38453698. Fax: 61-7-38453507. E-mail: david.mcmillan@qimr.edu.au.

† Present address: Centre for Immunology and Infection, Department of Biology, University of York and Hull York Medical School, YO10 5YW, United Kingdom.

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pothesized to contribute to changes in the disease association of GAS and GBS in the last few decades (1, 2, 5, 54, 58). Genome sequencing and other genetic studies have also reinforced the importance of mobile genetic elements (MGEs) in generating diversity within these species (5, 26, 37, 48, 51, 52). In fact, the pangenomes (representing the entire genetic repertoire of a species) of both GAS and GBS are considered to be open (51), implying that new genes continue to enter the population through ongoing interspecies LGT. In the absence of genomic sequences, our understanding of genomic variation in GGS is less clear. Given the close genetic relatedness between GAS, GBS, and GGS (24) and evidence that GGS genomes are more “chaotic” than GAS genomes (33), it is likely that the GGS pangenome is also open and is participating in interspecies LGT. In this regard, several studies have provided evidence for LGT involving GGS and GAS (27, 33). Our previous molecular epidemiological study also reported that intra- and interspecies LGT is likely to be occurring in environments where GGS and GAS are endemic (18, 19).

To date there has been little or no direct evidence for ICE mediated cross-species LGT between GGS and GAS or GGS and GBS. In the current study, we have provided the first detailed genetic and functional characterization of an ICE from GGS and demonstrate its conjugative transfer to both GAS and GBS. Significantly, ICES<sub>Sde3396</sub> contains a large internal recombinogenic region that carries functional genes and operons derived from both streptococcal and nonstreptococcal species, implying that it is a vehicle for dissemination of novel genes through the wider  $\beta$ -hemolytic streptococcal population.

#### MATERIALS AND METHODS

**Bacterial strains and molecular methods.** *S. dysgalactiae* subsp. *equisimilis* NS3396 was isolated from a patient presenting with pharyngitis and acute rheumatic fever (19). All other GGS, GAS, and GBS isolates (Table 1) were isolated from individuals presenting with streptococcal disease or as part of community surveys within Australia (17, 19, 20). The isolates used in this study are non-clonal, as determined by emm typing for GAS and GGS and serotyping for GBS. All streptococci were grown in Todd-Hewitt broth or on Todd-Hewitt agar supplemented with 2% horse blood. Growth medium was supplemented with kanamycin (500  $\mu$ g/ml) and/or streptomycin (400  $\mu$ g/ml) when appropriate.

Genomic DNA extraction, restriction enzyme digestions, ligations, PCR, and DNA sequencing all utilized standard procedures. Adapter PCR analysis was performed as previously described (18, 43). Briefly, genomic DNA was partially digested with a BclI and/or HindIII, purified, and ligated with a 5' phosphorylated oligonucleotide adaptor containing BclI or HindIII overhangs, respectively. Standard PCR was then undertaken, using one primer complementary to the adaptor sequence and another internal primer sequence specific for the DNA sequence of interest. The resulting products were purified after agarose gel electrophoresis and subject to DNA sequence analysis. The nucleotide (nt) sequence of adapter PCR primers is shown in Table 2.

**Determination of the ICES<sub>Sde3396</sub> nucleotide sequence.** A combination of long PCR (11), primer walking, and adapter PCR was employed to determine the complete sequence of ICES<sub>Sde3396</sub>. Primers used for these activities were based on DNA fragments identified by the initial genomic subtraction of NS3396 (19) or on the sequence of the putative GBS conjugative transposon present in the GBS 2603V/R genome (52). Forward and reverse DNA strand contigs were assembled using the Staden package. Assignment of open reading frames (ORFs) was achieved using the bacterial annotation system, BASys (53). The complete ICES<sub>Sde3396</sub> sequence is deposited in the GenBank database under accession number EU142041.

**PCR screening of streptococci.** The presence of ICES<sub>Sde3396</sub> and ICES<sub>Sa2603</sub> in GGS, GBS, and GAS was determined by PCR amplification of DNA representing three separate regions of ICES<sub>Sde3396</sub> (Fig. 1). The presence of R1 in streptococcal genomes was determined by amplification of a 3.1-kb fragment spanning from ICES<sub>Sde3396</sub>\_orf1 to ICES<sub>Sde3396</sub>\_orf5. The 4.2-kb amplicon used to identify the presence of the R2 region extends from ICES<sub>Sde3396</sub>\_orf17, within

TABLE 1. Bacterial strains used in this study

Isolate <sup>a</sup>	Emm type/serotype	Site of isolation	Source or reference
<b>GGS</b>			
NS3396	STG480	Throat	19
G120	STG4831	Throat	
G121	STC74	Unknown	19
G122	STC74	Unknown	19
GG510	STG6	Unknown	19
GG524	STG6	Unknown	19
GG548	STG4831	Unknown	19
MD013	STG10	Skin	This study
MD048	STG485	Skin	17
MD077	STG10	Skin	17
MD128 <sup>GCS</sup>	STG93464	Throat	17
MD172	STC74a	Skin	17
MD225	STC74a	Peritoneum	17
MD263	STC6979	Sputum	This study
MD284	STG11	Skin	17
MD323 <sup>GCS</sup>	STG62647	Blood	17
MD378	STG643	Skin	This study
MD409	STG10	Skin	This study
MD448	STG10	Skin	17
MD543	STG643	Skin	17
MD581	STC1400	Skin	This study
MD699	STG62647	Skin	17
MD813	STC74a	Blood	17
MD864	STG166b	Skin	17
MD894 <sup>GCS</sup>	STG62647	Urine	17
MD896	STG11	Skin	17
NS1121	STG4831	Unknown	19
NS383	New type	Blood	19
NS542	STG652	Blood	19
NS752	STG6	Blood	19
<b>GBS</b>			
RBH01	III	Vagina	This study
RBH02	Ib	Vagina	This study
RBH03	III	Vagina	This study
RBH04	1a/V	Vagina	This study
RBH05	V	Vagina	This study
RBH06	II	Vagina	This study
RBH07	Nontypeable	Vagina	This study
RBH08	Ia	Vagina	This study
RBH09	V	Vagina	This study
RBH10	V	Vagina	This study
RBH11	III	Vagina	This study
RBH12	V	Vagina	This study
RBH13	III	Vagina	This study
RBH14	Ib	Vagina	This study
RBH15	Nontypeable	Vagina	This study
RBH16	III	Vagina	This study
B36PS	IV	Vagina	This study
B37PD	V	Vagina	This study
50VD	1a	Vagina	This study
<b>GAS</b>			
NS282	st6030.1	Skin	20
NS344	1	Blood	20
NS351	58	Skin	20
NS414	11	Unknown	20
NS180	74	Throat	20
NS195	19	Blood	20
NS8	85	Blood	20
NS14	102	Skin	20
NS179	9.1	Skin	20
NS20	75.1	Blood	20
NS199	112	Throat	20
NS204	2	Blood	20
NS83	stNS554	Blood	20
NS205	56	Blood	20
NS210	22	Blood	20
NS225	99	Blood	20
NS226	4.2	Blood	20
NS235	24	Blood	20
NS236	77	Throat	20
NS240	st2904	Blood	20

<sup>a</sup> GGS isolates possessing the group C carbohydrate are indicated by a superscript GCS.

R1, to ICES<sub>Sde3396</sub>\_orf23 in R2. We chose to amplify a region extending from R1 to R2 to ensure that these regions were adjacent to one another in the streptococcal genomes examined. The R3 region was identified by amplification of a 3.2-kb fragment (ICES<sub>Sde3396</sub>\_orf55 to ICES<sub>Sde3396</sub>\_orf61). The nucleotide

TABLE 2. Primer combinations and expected amplicon sizes of the three *ICESde3396* regions targeted in the PCR screening of clinical GGS, GBS, and GAS isolates<sup>a</sup>

Primer	Nucleotide sequence (5' to 3')	Location <sup>a</sup>	Expected size (kb) <sup>a</sup>	Source or reference
R1F R1R	CTACTTTTTGTTGCCATTGG GAGTCAGTGTCCAAACTTTTCG	Amplification of the R1 region (orf1 to orf5) in <i>ICESde3396</i>	3.1	This study
R2F R2R	GTTTAAATCCAGATAAGCTACG ATCGATTATGATTTTGGTCAACG	Amplification of the R2 region (orf17 to orf23)	4.2	This study
R3F R3R	TTCCTACTTGACCAATACTGC GAGTGACTTAGTTATGAAGGACG	Amplification of the R3 region (orf55 to orf61)	3.2	This study
Adapter1 Adapter2	GATCCGCCTATAGTGAGTCGTATTAAC AGCTCGCCTATAGTGAGTCGTATTAAC	Adapter PCR		18, 43

<sup>a</sup> ORFs from *ICESde3396*.

sequences of primers used for PCR screening of the streptococcal isolates for the presence of R1, R2, and R3 regions are shown in Table 2.

**Conjugation of streptococci.** Filter mating experiments were carried out according to Smith and Guild (46). Prior to the mating, an antibiologically marked ICE was constructed by introducing a kanamycin resistance gene into *ICESde3396*. This was achieved by amplifying a 760-bp segment of *ICESde3396* extending from nt 15061 to nt 15820 with flanking *EcoRI* and *BamHI* restriction sites. Another 638-bp segment extending from nt 17305 to nt 17942 in the ICE was also amplified with flanking *BamHI* and *SpeHI* restriction sites. The two fragments were ligated through their *BamHI* sites, and the product was cloned into pSF152. A kanamycin resistance gene was subsequently cloned into the internal *BamHI* site, creating pSF152-ICE:Km. pSF152-ICE:Km was transformed into NS3396 using standard procedures (41) and recombinants (NS3396 *ICESde3396*:Km) selected on Todd-Hewitt agar containing 500 µg/ml kanamycin. Integration of the plasmid into the NS3396 genome was confirmed via PCR and Southern hybridization.

For all conjugations, NS3396 *ICESde3396*:Km was used as the donor strain. Spontaneous streptomycin-resistant GGS, GBS, and GAS isolates were used as recipient strains. Three milliliters of an overnight culture containing recipient organisms was supplemented with MgSO<sub>4</sub> (10 mM), bovine serum albumin (2 mg/ml), and DNase I (200 U). The donor culture (300 µl) was then added, and the entire bacterial culture was filtered onto nitrocellulose. After filtering, the membrane was placed cell side up on Todd-Hewitt agar, overlaid with 5 mm of 1.5% Todd-Hewitt agar, and incubated at 37°C overnight. The next day, the filter paper was retrieved, placed into a 50-ml tube containing Todd-Hewitt broth, and vortexed for 10 s. The resulting suspension was serially diluted in phosphate-buffered saline and plated onto Todd-Hewitt agar containing kanamycin and streptomycin. The conjugation frequency was determined by dividing the average number of transconjugants by the average number of donors for each experiment.

**Resistance to cadmium, arsenite, arsenate, and copper.** Overnight cultures of GGS and GBS were resuspended in Todd-Hewitt broth to an optical density at 600 nm (OD<sub>600</sub>) of 0.1. Aliquots of the cultures were supplemented with cadmium chloride (0 to 2 mM), sodium arsenate (0 to 7 mM), sodium arsenite (0 to 5 mM), or copper sulfate (0 to 7 mM) and grown at 37°C. The optical density of cultures was monitored at 600 nm. The OD<sub>600</sub> of cultures after 5 h of growth in the presence of arsenate, arsenite, or copper or 24 h of growth in the presence of cadmium are presented.

## RESULTS

**General features of *ICESde3396*.** We recently described genomic subtraction studies using pathovar (NS3396) and non-pathovar strains of GGS (19). A large proportion of the pathovar-specific DNA fragments identified in this study encoded partial genes with orthologues in GAS and/or GBS. While the majority of GAS-related DNA fragments were identified as being bacteriophage related (18, 19), the majority of the pathovar-specific GBS orthologues were related to an uncharacterized MGE found in GBS serotype V (2603V/R) (52). Through

a combination of long PCR, primer walking, and adapter PCR, we determined the full nucleotide sequence of this putative MGE in NS3396. *ICESde3396* was found to be approximately 64 kb in size and is predicted to contain 66 ORFs (Table 3). Only 38 of the ORFs are also found in the putative ICE in the GBS 2603V/R genome that we have designated *ICESa2603* (Fig. 1). The shared ORFs encode proteins involved in conjugal processes and include a site-specific tyrosine-like recombinase, relaxases, bacterial mobilization proteins, and TraG.

*ICESde3396* and *ICESa2603* also share a number of genes predicted to encode putative virulence factors. These include the abortive infection genes *abiGI* and *abiGII* which have a role in lactococcal phage exclusion (13), a putative surface-associated agglutinin receptor which may modulate adherence of streptococci to oral sites through binding of salivary agglutinin (7, 52), and several genes predicted to encode proteins conferring resistance to cadmium and copper. Like their lactococcal homologues, the *abiGI* and *abiGII* genes of *ICESde3396* and *ICESa2603* exhibit a lower percent GC content (31% and 34.6%, respectively) than GGS and GBS genomes (and the respective surrounding DNA within the ICE), suggesting that they were acquired by the ICE through LGT (38).

**Mosaic structure of an 18-kb region unique to *ICESde3396*.** The defining difference between *ICESde3396* and *ICESa2603* is the presence of an 18-kb internal region within *ICESde3396* (Fig. 1). The region encodes 21 ORFs (*ICESde3396orf19* to *orf40*) and is flanked by a small ORF with limited similarity to a transposase (*TnI545*) at one end, and a second putative transposase associated with the insertion sequence *IS1216* at the other. When the genes in this region were compared to those in the GenBank database, it became apparent that this region consists of four clusters whose orthologues are found in unrelated bacterial species, including nonstreptococcal species (Fig. 1).

The first of these clusters contains two genes from a cadmium resistance operon, *cadC* (*ICESde3396\_orf20*) and *cadD* (*ICESde3396\_orf21*), which is found in all GAS genomes, in some but not all GBS genomes, and in *Streptococcus gordonii* and *Streptococcus parasanguinis*. *cadD* orthologues (but not *cadC*) are also found in *Neisseria meningitidis* and *Neisseria gonorrhoeae*. The five genes in the second cluster are predicted to encode two putative recombinases, a truncated lysin, a cell

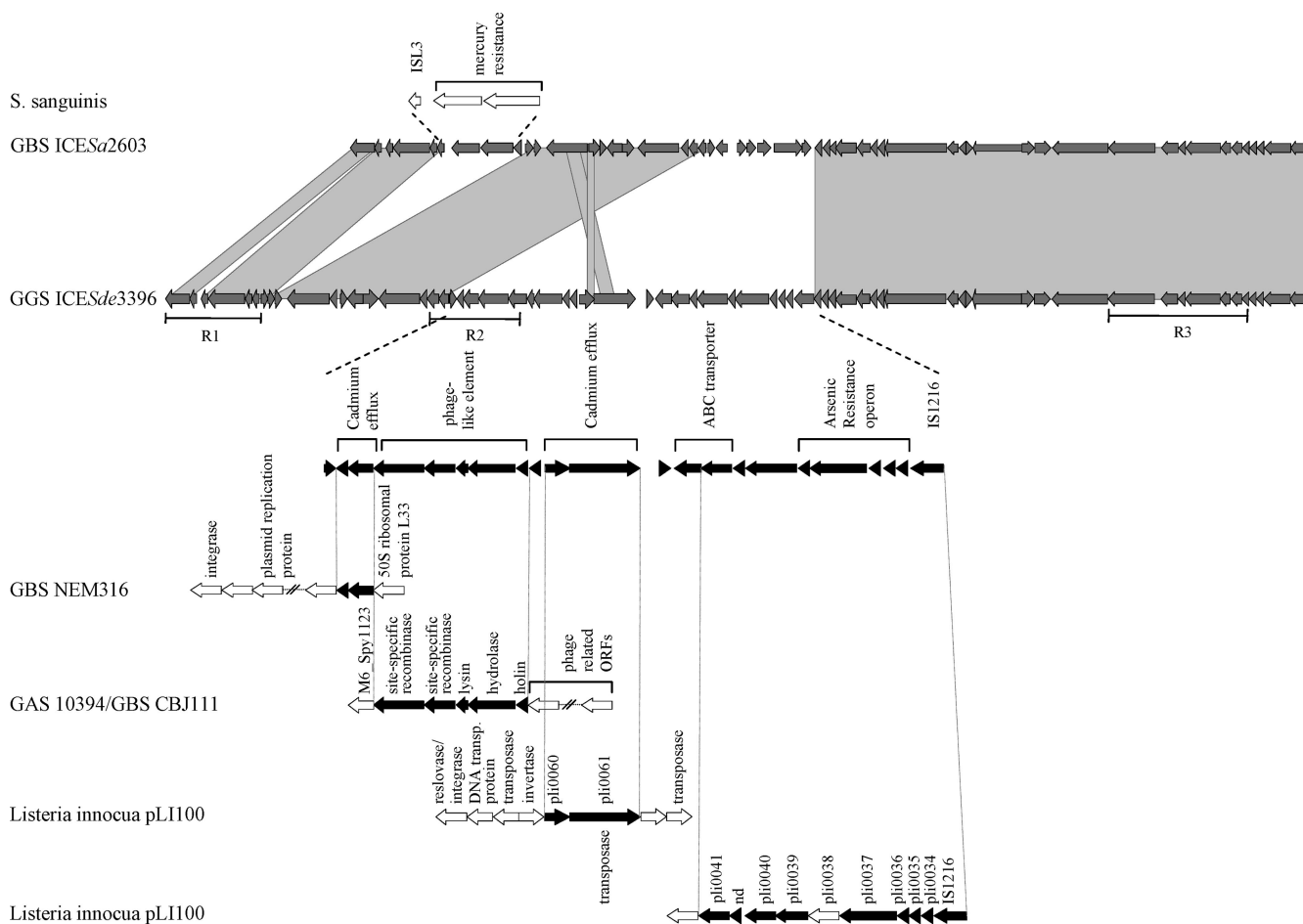


FIG. 1. Genetic organization and protein alignment of *ICESde3396* with *ICESa2603*. Individual ORFs are represented by block arrows. Conserved proteins exhibiting greater than 45% amino acid identity between *ICESde3396* and *ICESa2603* are represented by the shading. The *ICESde3396* genome was annotated using the bacterial annotation system BASys (53) and comparisons performed using the Artemis Comparison Tool (10). ORFs in the 18.1-kb recombinogenic region are depicted by block arrows. The four gene clusters in the 18-kb recombinogenic region and corresponding regions in other bacterial genomes are indicated by dashed lines. The R1, R2, and R3 regions are also shown.

wall hydrolase, and holin with similarity at the amino acid level to ORFs from a GAS bacteriophage (3, 4) and GBS MGE. The low amino acid sequence identity (<70%) and absence of significant nucleotide sequence homology suggest a distant evolutionary relationship between these orthologues. The third cluster (*ICESde3396\_orf28* and *ICESde3396\_orf29*) encodes a second cadmium resistance operon lacking any sequence homology to the previously described cadmium resistance operon. The orthologues of this operon are found on plasmids from *Listeria innocua* (pLI100) and *Lactococcus lactis* (50) and a genomic island from *Streptococcus thermophilus* (40). The final cluster contains genes whose orthologues are found in a different region of the pLI100 plasmid. These include genes from a *yadG*- and *yadH*-like operon that also has orthologues in *Enterococcus faecalis*. The remaining seven genes of this cluster form a putative arsenic resistance operon. While the overall amino acid identity between the ORFs in this operon and their corresponding homologues from pLI100 is high (>98%), a truncation in the purported arsenic transporter (*ArsB*) and absence of an *ArsC* homologue suggests the operon is nonfunctional.

To provide a better understanding of how this recombinogenic region may have evolved, we undertook a genomic examination of each of the four clusters and surrounding DNA in their purported progenitor chromosomes. In each case, the cluster was situated in close proximity to MGE-associated genes, suggesting that incorporation of these elements into *ICESde3396* has occurred through standard LGT (Fig. 1). The observation that the individual clusters (e.g., the two cadmium clusters) are also present in other unrelated bacterial species provides further support for the role of MGEs in their lateral dissemination.

**The chromosomal location of *ICESde3396* is conserved.** In order to determine the flanking region of *ICESde3396* in NS3396, adaptor PCRs using proximal *ICESde3396*-specific primers in conjunction with an adaptor-specific primer were performed (43). Sequencing of the PCR products flanking *ICESde3396\_orf66* identified two genes. The first of these genes encodes a small ORF with significant homology to a small hypothetical protein (SAG1300) adjacent to *ICESa2603* in the 2603V/R genome (52). The second ORF was homologous to the 50s ribosomal gene L7/L12 and is also found in

TABLE 3. Features and predicted ORF functions of ICES<sub>Sde3396</sub>

ORF	Position (bp)		No. of aa	Direction of DNA strand	% G/C content	% aa identity	Species and gene no./locus <sup>f</sup>	Predicted function
	Start	Stop						
1	1212	1	403	-	32.34	100	SAG1247	Site-specific recombinase (tyrosine-like)
2	1450	1223	75	-	34.65	100	SAG1248	Hypothetical
3	1953	1729	74	-	33.78	100	SAG1249	Transcriptional regulator, Cro/CI family
4	3999	2134	621	-	32.05	99	SAG1250	Relaxase
5	4351	3986	121	-	34.43	100	SAG1251	Bacterial mobilization protein (MobC)
6	4720	4358	120	-	34.71	100	SAG1252	Hypothetical
7	5043	5150	35	+	26.85	100	SAJ_1275	Hypothetical
8	5211	5693	160	+	39.54	100	SAJ_1276	Hypothetical
9	5534	5875	113	+	38.60	93 <sup>a</sup>	<i>S. suis</i> 89/1591 <sup>c</sup>	Transposase/integrase
10	8279	6150	709	-	37.61	100	SAG1257	Probable cadmium efflux ATPase CadA
11	8640	8272	122	-	33.60	99	SAG1258	Cadmium efflux system accessory protein CadC
12	8843	9142	99	+	36.33	100	SAG1259	Hypothetical
13	9984	9196	262	-	30.29	100	SAG1260	Hypothetical
14	9990	10589	199	+	42.50	100	SAG1261	Hypothetical membrane-spanning protein
15	12850	10763	695	-	43.92	98	SAG1262	Probable copper exporting ATPase TcrB-like
16	13195	12989	68	-	36.23	98	GBS 2603V/R <sup>c,d</sup>	Copper-transporting ATPase TcrZ-like
17	13819	13343	158	-	42.77	96 <sup>a</sup>	SAG1263	Copper-transporting ATPase TcrA-like
18	14252	13806	148	-	36.24	92	SAG1264	Transcriptional repressor TcrY-like
19	14460	14552	30	+	40.86			ICES <sub>Sde3396</sub> _orf19
20	15048	14710	112	-	32.74	99	SAI2183 <sup>c</sup>	Cadmium efflux system accessory protein CadC
21	15717	15061	218	-	33.49	99	SAI2182 <sup>c</sup>	Cadmium resistance protein CadD
22	17311	15749	520	-	37.56	71	M6_Spy1124	Site-specific recombinase
23	18222	17311	303	-	37.50	67	M6_Spy1125	Site-specific recombinase
24	18499	18338	53	-	30.86	58	TnJ207.3orf55	Phage lysin
25	20077	18608	489	-	43.06	59	SAM_0643	Amidase/phage cell wall hydrolase
26	20479	20084	131	-	34.09	66 <sup>a</sup>	SAM_0642	Holin
27	20663	20457	68	-	36.71			ICES <sub>Sde3396</sub> _orf27
28	21300	21659	119	+	30.83	99	<i>L. innocua</i> pli0060 <sup>c</sup>	Cadmium efflux system accessory protein CadC
29	21656	23773	705	+	37.11	100	<i>L. innocua</i> pli0061 <sup>c</sup>	Cadmium efflux ATPase CadA
30	24476	24706	76	+	32.47			ICES <sub>Sde3396</sub> _orf30
31	25589	24819	256	-	32.94	90	<i>E. faecalis</i> V583 EF3208 <sup>c</sup>	Inner membrane transport permease YadH-like
32	26517	25582	311	-	35.69	97 <sup>a</sup>	<i>L. innocua</i> pli0041	ABC transporter, ATP subunit YadG-like
33	26782	26672	36	-	35.14	99 <sup>d</sup>	<i>L. innocua</i> pLI100	ND <sup>b</sup>
34	28480	26807	557	-	37.51	99	<i>L. innocua</i> pli0040	Coenzyme A disulfide reductase LpdA
35	28713	28501	70	-	37.09	100 <sup>a</sup>	<i>L. innocua</i> pli0039	Arsenical resistance protein ACR3-like (ArsB)
36	30510	28768	580	-	37.81	99	<i>L. innocua</i> pli0037	Arsenical pump-driving ATPase ArsA
37	30893	30546	115	-	36.21	98	<i>L. innocua</i> pli0036	Arsenical resistance repressor ArsR
38	31398	31027	123	-	38.98	98	<i>L. innocua</i> pli0035	Arsenical resistance trans-acting repressor ArsD
39	31666	31400	88	-	32.96	98	<i>L. innocua</i> pli0034	Arsenical resistance repressor ArsR
40	32558	31875	227	-	37.43	98	<i>L. innocua</i> pli0032	Transposase (IS1216)
41	33200	32841	119	-	34.17	77	SAG1273	Hypothetical
42	33576	33187	129	-	36.15	97	SAG1274	Hypothetical
43	33800	33573	75	-	38.60	96	SAG1275	Hypothetical
44	34930	33854	358	-	38.25	91	SAG1276	Hypothetical zinc finger protein
45	35608	34970	212	-	39.44	95	SAI1373	Hypothetical
46	35994	35704	96	-	32.30	91	SAG1278	Hypothetical
47	36307	36008	99	-	42.33	84	SAG1279	Hypothetical
48	43214	36378	2278	-	41.00	99	SAG1280	SNF2-related helicase
49	43802	43251	183	-	38.04	100	SAG1281	Hypothetical
50	43977	43786	63	-	42.71	100	SAG1282	Calcium-binding protein
51	46772	46882	36	+	41.44	100 <sup>d</sup>	GBS 2603V/R	ND
52	48873	43978	1631	-	41.71	99	SAG1283	Agglutinin receptor precursor
53	49140	49742	200	+	34.66	100	SAG1284	Abortive infection protein AbiGI
54	49739	50584	281	+	31.80	100	SAG1285	Abortive infection protein AbiGII
55	53471	50670	933	-	42.68	93	SAG1286	<i>N</i> -acetylmuramoyl-L-alanine amidase
56	55824	53473	783	-	40.43	99	SAG1287	Hypothetical
57	57050	56196	284	-	41.75	99	SAG1289	Hypothetical
58	57323	57069	84	-	44.31	100	SAG1290	Hypothetical
59	59146	57329	605	-	40.98	99	SAG1291	Conjugal transfer protein TraG
60	59670	59146	174	-	39.24	99	SAG1292	Hypothetical
61	60301	59711	196	-	39.26	100	SAG1293	Putative protease
62	60537	60298	79	-	35.42	100	SAG1294	Hypothetical
63	60929	60540	129	-	41.03	100	SAG1295	Hypothetical (potential arsenate reductase)
64	61362	60934	142	-	38.46	100	SAG1296	Hypothetical
65	62701	61346	451	-	44.32	99	SAG1297	DNA methylase
66	63668	62849	272	-	40.42	100	SAG1299	Replication initiator

<sup>a</sup> Proteins with partial homology to the GenBank database that may represent truncated proteins through a frameshift or deletion events.

<sup>b</sup> ORFs with less than 35% amino acid identity to the GenBank database are defined as having no database homologue (ND).

<sup>c</sup> These ORFs had equal identity to multiple ORFs from the same/different isolate(s).

<sup>d</sup> Homology is based on BlastN analysis and represents a nonannotated ORF in the GenBank database.

<sup>e</sup> aa, amino acid(s).

<sup>f</sup> Streptococcal species unless indicated otherwise.

proximity to ICES<sub>Sa2603</sub>. Subsequent PCR analysis confirmed that the 50s ribosomal L7/L12 gene flanked the ICES in all ICE-positive GGS and GBS strains examined in this study (data not shown). The similar flanking sequences suggested

that integration occurs via a specific mechanism common to the tyrosine family of recombinases (8).

**Distribution of ICES<sub>Sde3396</sub> and ICES<sub>Sa2603</sub> in β-hemolytic streptococci.** The presence of ICES<sub>Sde3396</sub>-like and ICES<sub>Sa2603</sub>-

TABLE 4. Distribution of R1, R2, and R3 regions in GGS, GBS, and GAS isolates

Regions <sup>a</sup>	GGS (n = 30)	GBS (n = 19)	GAS (n = 20)
R1 <sup>-</sup> R2 <sup>-</sup> R3 <sup>-</sup>	15	10	20
R1 <sup>+</sup> R2 <sup>+</sup> R3 <sup>+</sup>	4	7	0
R1 <sup>+</sup> R2 <sup>-</sup> R3 <sup>+</sup>	2	1	0
R1 <sup>+</sup> R2 <sup>+</sup> R3 <sup>V</sup>	2	0	0
R1 <sup>-</sup> R2 <sup>+</sup> R3 <sup>+</sup>	2	0	0
R1 <sup>-</sup> R2 <sup>-</sup> R3 <sup>+</sup>	4	0	0
R1 <sup>+</sup> R2 <sup>+</sup> R3 <sup>-</sup>	1	0	0
R1 <sup>+</sup> R2 <sup>V</sup> R3 <sup>-</sup>	0	1	0

<sup>a</sup> V, a PCR product was generated for this region. However, the size of this product differed from that observed for the corresponding fragment in ICES<sub>Sde3396</sub>.

like elements in other GGS, GBS, and GAS strains was examined using PCR that targeted three independent regions of ICES<sub>Sde3396</sub> (Fig. 1). The first product (R1) extended from ICES<sub>Sde3396\_orf1</sub> to ICES<sub>Sde3396\_orf5</sub>. The second product (R2) extended from ICES<sub>Sde3396\_orf17</sub> to ICES<sub>Sde3396\_orf23</sub> and includes DNA common to both ICES<sub>Sde3396</sub> and ICES<sub>Sa2603</sub>, as well as DNA unique to ICES<sub>Sde3396</sub> (i.e., the 18-kb chimeric region). Amplification of this fragment enabled this differentiation between strains harboring ICES<sub>Sde3396</sub>-like and ICES<sub>Sa2603</sub>-like MGEs and also confirmed the proximal locations of these regions in ICES<sub>Sde3396</sub>-like ICEs. The third fragment (R3) spanned ICES<sub>Sde3396\_orf55</sub> to ICES<sub>Sde3396\_orf61</sub>.

Of the 69 β-hemolytic isolates screened, 11 were positive for all three regions (i.e., R1<sup>+</sup> R2<sup>+</sup> R3<sup>+</sup>), implying that they possessed ICES<sub>Sde3396</sub>-like elements (Table 4). Four of the 11 positive isolates were GGS, and the remainder were GBS. Another four isolates (three GGS and one GBS) possessed both the R1 and R3 regions but lacked the R2 region (i.e., R1<sup>+</sup> R2<sup>-</sup> R3<sup>+</sup>), suggesting that they harbored ICES<sub>Sa2603</sub>-like elements. The difference in the proportion of strains that were R1<sup>+</sup> R2<sup>+</sup> R3<sup>+</sup> or R1<sup>+</sup> R2<sup>-</sup> R3<sup>+</sup> was not found to be statistically significant, as determined by Fisher's exact test. The diagnostic PCR also identified nine GGS isolates, and one GBS isolate possessed at least one of the three regions used to determine the presence of the ICEs but did not conform to the PCR profile used to define ICES<sub>Sde3396</sub>-like (R1<sup>+</sup> R2<sup>+</sup> R3<sup>+</sup>) or ICES<sub>Sa2603</sub>-like (R1<sup>+</sup> R2<sup>-</sup> R3<sup>+</sup>) elements. Of note, none of the GAS isolates screened were positive for any of the three regions.

#### ICES<sub>Sde3396</sub> confers resistance to cadmium and arsenate.

To test whether the metal resistance operons identified within ICES<sub>Sde3396</sub> were functional, NS3396 (R1<sup>+</sup> R2<sup>+</sup> R3<sup>+</sup>), GGS10 (R1<sup>-</sup> R2<sup>-</sup> R3<sup>-</sup>), GBS RBH01 (R1<sup>+</sup> R2<sup>+</sup> R3<sup>+</sup>), and GBS RBH03 (R1<sup>-</sup> R2<sup>-</sup> R3<sup>-</sup>) were grown in Todd-Hewitt broth supplemented with increasing concentrations of sodium(III) arsenite, sodium(V) arsenate, copper chloride, or cadmium chloride, and optical density of the cultures was measured at regular intervals. Whereas growth of GGS10 was inhibited by 1 mM arsenate, both NS3396 and GBS RBH01 showed little or no growth inhibition in 5 mM arsenate. In contrast to the ICE-lacking strains, both ICE-positive strains possessed an increased tolerance to cadmium at concentrations below 1 mM (Fig. 2). No association between the presence of ICE<sub>Sde3396</sub>-like elements and arsenite or copper resistance was observed.

To provide additional support for a correlation between the tolerance to cadmium and arsenate and the presence of ICES<sub>Sde3396</sub>-like elements, additional GGS and GBS strains that were R1<sup>+</sup> R2<sup>+</sup> R3<sup>+</sup>, R1<sup>+</sup> R2<sup>-</sup> R3<sup>+</sup>, or R1<sup>-</sup> R2<sup>-</sup> R3<sup>-</sup> were grown in the presence of 3 mM arsenate or 0.4 mM cadmium chloride (Fig. 3). Four of the five R1<sup>+</sup> R2<sup>+</sup> R3<sup>+</sup> isolates were capable of growing in 3 mM arsenate. In contrast, none of the R1<sup>+</sup> R2<sup>-</sup> R3<sup>+</sup> isolates were able to grow in the presence of arsenate. With the exception of a single strain, all ICE-harboring isolates were also able to grow in the presence of cadmium. None of the six ICE-negative strains grew in the presence of arsenate or cadmium.

**ICES<sub>Sde3396</sub> is transferable to other β-hemolytic streptococci.** As the nucleotide sequence data suggested that ICES<sub>Sde3396</sub> has the full complement of genes necessary for conjugation, we next investigated whether we could transfer this element to other β-hemolytic streptococci. To do so we marked ICES<sub>Sde3396</sub> by replacing a 1.5-kb segment within the 18-kb recombinogenic region with a gene conferring kanamycin resistance. We reasoned that as the 18-kb recombinogenic region was absent in ICES<sub>Sa2603</sub>, it would not contain any genes necessary for conjugal processes. After filter mating between NS3396 ICES<sub>Sde3396</sub>:Km and GGS10, doubly antibiotic-

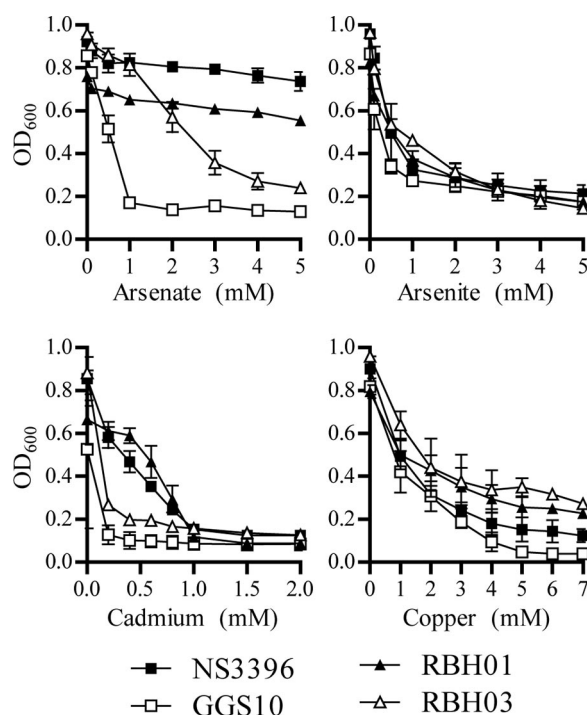


FIG. 2. Growth of GGS and GBS in the presence of arsenate, arsenite, cadmium, and copper. GGS strains are represented by squares and GBS strains by triangles. Closed symbols represent isolates containing ICES<sub>Sde3396</sub> or ICE ICES<sub>Sde3396</sub>-like elements (i.e., R1<sup>+</sup> R2<sup>+</sup> R3<sup>+</sup>). Open symbols represent ICES<sub>Sde3396</sub>-negative strains (i.e., R1<sup>-</sup> R2<sup>-</sup> R3<sup>-</sup>). Strains were grown overnight in Todd-Hewitt broth, harvested, and resuspended medium containing increasing concentrations of sodium arsenate, sodium arsenite, cadmium chloride, or copper chloride. The OD<sub>600</sub> of cultures was measured at regular intervals during the growth cycle. Data is presented as the OD<sub>600</sub> after 24 h of growth in the presence of cadmium and 5 h of growth in the presence of arsenate, arsenite, and copper.

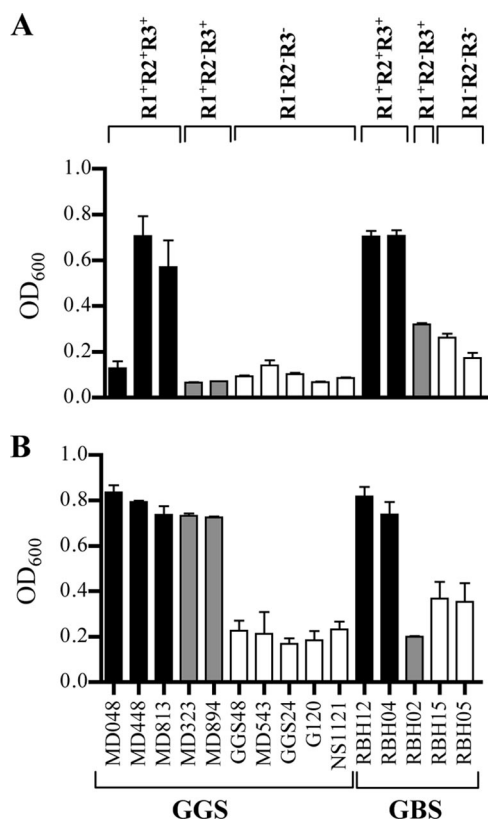


FIG. 3. ICE*Sde*3396 is associated with increased resistance to arsenate and cadmium. GGS and GBS were grown in the presence of 3 mM sodium arsenate (A) or 0.4 mM cadmium chloride. (B) Black bars represent isolates containing ICE*Sde*3396-like elements (i.e., R1<sup>+</sup>R2<sup>+</sup>R3<sup>+</sup>). Gray bars represent isolates containing ICE*Sa*2603-like elements (i.e., R1<sup>+</sup>R2<sup>+</sup>R3<sup>+</sup>). Open bars represent ICE*Sde*3396-negative strains (i.e., R1<sup>-</sup>R2<sup>-</sup>R3<sup>-</sup>). Data is presented as the OD<sub>600</sub> after 5 h of growth in the presence of arsenate and 24 h of growth in the presence of cadmium.

resistant GGS recipients (i.e., kanamycin and streptomycin resistant) were observed on Todd-Hewitt agar, suggesting that transfer of the ICE had occurred. Subsequently, we successfully transferred the ICE to GAS NS344 and GBS RBH05. The frequency of mobilization of ICE*Sde*3396:Km into GGS, GBS, and GAS was found to be  $2 \times 10^{-4}$ ,  $6 \times 10^{-6}$ , and  $1.5 \times 10^{-3}$ , respectively. Compared to their parental strains, all transconjugants were also found to have increased tolerance to arsenate and cadmium (Fig. 4). As the kanamycin resistance marker was incorporated into the 18-kb recombinogenic region, it was conceivable that this region alone may be incorporated into recipient strains through the resident IS*1216* transposase. To test for this possibility, five GAS, GBS, and GGS transconjugants were chosen at random, and PCR analysis using the diagnostic primers specific for the genes in the R1, R2, and R3 regions was undertaken. All transconjugants tested possessed all three regions (data not shown).

## DISCUSSION

MGEs enable the nonvertical transfer of DNA between bacterial species. As such, they provide vehicles for rapid microbial adaptation and evolution. Previous studies have demon-

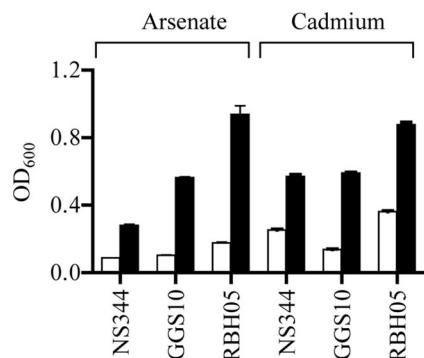


FIG. 4. Growth of ICE3396-negative and ICE3396-positive transconjugants in the presence of 0.4 mM cadmium chloride and 3 mM sodium arsenate. Parental strains are represented by the open bars. Transconjugants harboring ICE*Sde*3396 are represented by black bars. Data is presented as the mean OD<sub>600</sub> of cultures grown for 5 h in the presence of arsenate and 24 h in the presence of cadmium.

strated MGEs are present in GAS, GBS, and GGS genomes and that many virulence factors are associated also with MGEs in these species (25, 45, 52). Bacteriophages are the most common MGE found in GAS genomes. All GAS strains analyzed to date have at least one prophage sequence, and most are polylysogenic (25). In contrast, bacteriophages appear to be much rarer in GBS and ICES more prevalent. Without a genomic sequence, our understanding of the factors that influence genetic variation of GGS is less clear. Our previous study (18) identified a bacteriophage in NS3396 with significant homology to a bacteriophage from GAS. In the current study, we identified an ICE within the same GGS isolate that shares significant homology with a putative ICE in GBS. Thus, GGS appears capable of receiving and/or donating DNA to both these pathogenic species.

ICE*Sde*3396 carries genes whose orthologues can be found in numerous bacterial species, including GBS, GAS, *S. parasanguinis*, *Streptococcus suis*, *Enterococcus* spp., *Lactococcus* spp., *Listeria* spp., and *Neisseria* spp. Genetic analysis of the hypothesized progenitor chromosomes of the four independent clusters in the 18-kb recombinogenic region of ICE*Sde*3396 demonstrated that each was flanked by MGE-associated genes, indicating that the accumulation of these clusters within ICE*Sde*3396 occurs through typical LGT events. Additional transposase/integrases are also found in other parts of ICE*Sde*3396 and ICE*Sa*2603. An earlier study by Tettelin et al. suggested that both the GAS and GBS pangenomes were open (51). We hypothesize that as GGS is closely related to these species, its genome is also open, and that ICE*Sde*3396 is one element that contributes to the open genomes of  $\beta$ -hemolytic streptococci. The ICE serves as a repository for genes from diverse bacterial origins; incorporation of these genes within the ICE also prevents disruption of chromosomally encoded genes whose functions may be critical to bacterial fitness. Additionally, the ICE is a vehicle that enables dissemination of these newly acquired genes through the  $\beta$ -hemolytic population.

Our functional studies demonstrated that four of the five isolates harboring ICE*Sde*3396-like elements possessed an increased resistance to arsenate. Similarly, six of seven isolates harboring the ICE*Sde*3396-like or ICE*Sa*2603-like element

had increased tolerances to cadmium. The association between the presence of the ICEs and tolerance to these metals and the fact that these tolerances were transferred to recipient strains provides strong evidence that these phenotypic properties are carried on the MGEs. We attribute the lack of cadmium resistance in GBS RBH02 and arsenate resistance in GGS MD048 to either ongoing modular evolution of the ICE or mutations resulting in a loss of phenotype. Modular evolution is a hallmark of all MGEs (28), and the cadmium resistance operon common to ICES<sub>Sde3396</sub> and ICES<sub>Sa2603</sub> lies adjacent to a putative transposase/integrase. Transposition events involving this element may have resulted in the loss or mutation of the cadmium operon region in GGS MD048. This is supported by the data demonstrating that the locus was amplifiable from ICES<sub>Sde3396</sub> in NS3396 but not the ICE in MD048. The archetypal arsenic resistance operons contain three genes, *arsR*, *arsB*, and *arsC* (44). As the *ars* operon in the ICES<sub>Sde3396</sub> includes a truncated *arsB* and lacks an *arsC* homologue, we were surprised to find the operon to be functional. As there is no apparent advantage to possession of this operon, further mutations accumulating in this region (deletions or point mutations) may eventually render the operon nonfunctional.

Although ICES<sub>Sde3396</sub> does not carry antibiotic resistance genes, it does contain several genes whose homologues are found in other MGEs that do harbor antibiotic-resistant determinants, suggesting that transfer of DNA between these elements can occur. As an example, ICES<sub>Sde3396</sub> contains homologues of genes found in a *mefA* carrying MGE in GAS 10394. More strikingly, a transposase associated with IS1216 is present at one end of the 18-kb chimeric region. IS1216 is part of a promiscuous insertion element that in other bacterial species carries genes conferring resistance to vancomycin (32, 39), erythromycin (32), and chloramphenicol (55). An IS1216 element from *Enterococcus hirae* also carries genes encoding a low affinity penicillin binding protein (42). Penicillin has remained the drug of choice for treatment and prevention of  $\beta$ -hemolytic infections for over 50 years. Unlike related bacteria, the  $\beta$ -hemolytic streptococci have not developed resistance to penicillin; the reasons for this remain unknown (31). As penicillin resistance has not arisen naturally in  $\beta$ -hemolytic streptococci, even in the face of long-term penicillin treatment, mutation of existing genes seems to be an unlikely source of future resistance. Rather, we speculate that transfer of penicillin resistance genes via an ICE is more likely to provide a mechanism for the acquisition of penicillin (or other antibiotic) resistance phenotypes. In this regard, tetracycline resistance genes linked to IS1216 have recently been reported in GGS (36). As antibiotic resistance increases in other bacterial species found in the same environmental niches occupied by  $\beta$ -hemolytic streptococci, the probability of transfer of antibiotic resistance determinants can only increase.

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#### ADDENDUM IN PROOF

After the manuscript was accepted, we found that RBH02 (Fig. 3) did not yield PCR products of any size. Consequently, its expected genotype is R1<sup>-</sup> R2<sup>-</sup> R3<sup>-</sup>, which in fact correlates with its resistance phenotype. This change does not affect our conclusions.

#### REFERENCES

- Aziz, R. K., R. A. Edwards, W. W. Taylor, D. E. Low, A. McGeer, and M. Koth. 2005. Mosaic prophages with horizontally acquired genes account for the emergence and diversification of the globally disseminated MIT1 clone of *Streptococcus pyogenes*. *J. Bacteriol.* **187**:3311–3318.
- Banks, D. J., S. B. Beres, and J. M. Musser. 2002. The fundamental contribution of phages to GAS evolution, genome diversification and strain emergence. *Trends Microbiol.* **10**:515–521.
- Banks, D. J., S. F. Porcella, K. D. Barbian, S. B. Beres, L. E. Philips, J. M. Voyich, F. R. DeLeo, J. M. Martin, G. A. Somerville, and J. M. Musser. 2004. Progress toward characterization of the group A *Streptococcus* metagenome: complete genome sequence of a macrolide-resistant serotype M6 strain. *J. Infect. Dis.* **190**:727–738.
- Banks, D. J., S. F. Porcella, K. D. Barbian, J. M. Martin, and J. M. Musser. 2003. Structure and distribution of an unusual chimeric genetic element encoding macrolide resistance in phylogenetically diverse clones of group A *Streptococcus*. *J. Infect. Dis.* **188**:1898–1908.
- Beres, S. B., and J. M. Musser. 2007. Contribution of exogenous genetic elements to the group A *Streptococcus* metagenome. *PLoS ONE* **2**:e800.
- Bisno, A. L., F. A. Rubin, P. P. Cleary, and J. B. Dale. 2005. Prospects for a group A streptococcal vaccine: rationale, feasibility, and obstacles—report of a National Institute of Allergy and Infectious Diseases workshop. *Clin. Infect. Dis.* **41**:1150–1156.
- Brady, L. J., D. A. Piacentini, P. J. Crowley, P. C. Oyston, and A. S. Bleiweis. 1992. Differentiation of salivary agglutinin-mediated adherence and aggregation of mutans streptococci by use of monoclonal antibodies against the major surface adhesin P1. *Infect. Immun.* **60**:1008–1017.
- Burrus, V., G. Pavlovic, B. Decaris, and G. Guedon. 2002. Conjugative transposons: the tip of the iceberg. *Mol. Microbiol.* **46**:601–610.
- Burrus, V., and M. K. Waldor. 2004. Shaping bacterial genomes with integrative and conjugative elements. *Res. Microbiol.* **155**:376–386.
- Carver, T. J., K. M. Rutherford, M. Berriman, M. A. Rajandream, B. G. Barrell, and J. Parkhill. 2005. ACT: the Artemis Comparison Tool. *Bioinformatics* **21**:3422–3423.
- Cheng, S., C. Fockler, W. M. Barnes, and R. Higuchi. 1994. Effective amplification of long targets from cloned inserts and human genomic DNA. *Proc. Natl. Acad. Sci. USA* **91**:5695–5699.
- Chhatwal, G. S., D. J. McMillan, and S. R. Talay. 2006. Pathogenicity factors in Group C and G streptococci, p. 213–221. *In* V. A. Fischetti, R. P. Novick, J. J. Ferretti, D. A. Portnoy, and J. I. Rood (ed.), *Gram-positive pathogens*, 2nd ed. ASM Press, Washington, DC.
- Chopin, M. C., A. Chopin, and E. Bidnenko. 2005. Phage abortive infection in lactococci: variations on a theme. *Curr. Opin. Microbiol.* **8**:473–479.
- Cohen-Poradosu, R., J. Jaffe, D. Lavi, S. Grisariu-Greenzaid, R. Nir-Paz, L. Valinsky, M. Dan-Goor, C. Block, B. Beall, and A. E. Moses. 2004. Group G streptococcal bacteremia in Jerusalem. *Emerg. Infect. Dis.* **10**:1455–1460.
- Cone, L. A., D. R. Woodard, P. M. Schlievert, and G. S. Tomory. 1987. Clinical and bacteriologic observations of a toxic shock-like syndrome due to *Streptococcus pyogenes*. *N. Engl. J. Med.* **317**:146–149.
- Cunningham, M. W. 2000. Pathogenesis of group A streptococcal infections. *Clin. Microbiol. Rev.* **13**:470–511.
- Davies, M. R., D. J. McMillan, R. G. Beiko, V. Barroso, R. Geffers, K. S. Sriprakash, and G. S. Chhatwal. 2007. Virulence profiling of *Streptococcus dysgalactiae* subspecies *equisimilis* isolated from infected humans reveals 2 distinct genetic lineages that do not segregate with their phenotypes or propensity to cause diseases. *Clin. Infect. Dis.* **44**:1442–1454.
- Davies, M. R., D. J. McMillan, G. H. Van Domselaar, M. K. Jones, and K. S. Sriprakash. 2007. Phage 3396 from a *Streptococcus dysgalactiae* subsp. *equisimilis* pathovar may have its origins in *Streptococcus pyogenes*. *J. Bacteriol.* **189**:2646–2652.
- Davies, M. R., T. N. Tran, D. J. McMillan, D. L. Gardiner, B. J. Currie, and K. S. Sriprakash. 2005. Inter-species genetic movement may blur the epidemiology of streptococcal diseases in endemic regions. *Microbes Infect.* **7**:1128–1138.
- Delvecchio, A., B. J. Currie, J. D. McArthur, M. J. Walker, and K. S. Sriprakash. 2002. *Streptococcus pyogenes prtFII*, but not *sfbl*, *sfblI* or *flp54*, is represented more frequently among invasive-disease isolates of tropical Australia. *Epidemiol. Infect.* **128**:391–396.
- Dermer, P., C. Lee, J. Eggert, and B. Few. 2004. A history of neonatal group



- B streptococcus with its related morbidity and mortality rates in the United States. *J. Pediatr. Nurs.* **19**:357–363.
22. **Efstratiou, A.** 1997. Pyogenic streptococci of Lancefield groups C and G as pathogens in man. *Soc. Appl. Bacteriol. Symp. Ser.* **26**:S72–S79.
  23. **Ekelund, K., P. Skinhoj, J. Madsen, and H. B. Konradsen.** 2005. Invasive group A, B, C and G streptococcal infections in Denmark 1999–2002: epidemiological and clinical aspects. *Clin. Microbiol. Infect.* **11**:569–576.
  24. **Facklam, R.** 2002. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin. Microbiol. Rev.* **15**:613–630.
  25. **Ferretti, J. J., D. Ajdic, and W. M. McShan.** 2004. Comparative genomics of streptococcal species. *Indian J. Med. Res.* **119**(Suppl.):1–6.
  26. **Ferretti, J. J., W. M. McShan, D. Ajdic, D. J. Savic, G. Savic, K. Lyon, C. Primeaux, S. Sezate, A. N. Suvorov, S. Kenton, H. S. Lai, S. P. Lin, Y. Qian, H. G. Jia, F. Z. Najjar, Q. Ren, H. Zhu, L. Song, J. White, X. Yuan, S. W. Clifton, B. A. Roe, and R. McLaughlin.** 2001. Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. *Proc. Natl. Acad. Sci. USA* **98**:4658–4663.
  27. **Franken, C., G. Haase, C. Brandt, J. Weber-Heynemann, S. Martin, C. Lammler, A. Podbielski, R. Luttkien, and B. Spellerberg.** 2001. Horizontal gene transfer and host specificity of beta-haemolytic streptococci: the role of a putative composite transposon containing *scpB* and *lmb*. *Mol. Microbiol.* **41**:925–935.
  28. **Frost, L. S., R. Leplae, A. O. Summers, and A. Toussaint.** 2005. Mobile genetic elements: the agents of open source evolution. *Nat. Rev. Microbiol.* **3**:722–732.
  29. **Gaviria, J. M., and A. L. Bisno.** 2000. Group C and G streptococci, p. 238–254. *In* D. L. Stevens and E. L. Kaplan (ed.), *Streptococcal infections: clinical aspects, microbiology, and molecular pathogenesis*. Oxford University Press, Oxford, United Kingdom.
  30. **Hindsholm, M., and H. C. Schonheyder.** 2002. Clinical presentation and outcome of bacteraemia caused by beta-haemolytic streptococci serogroup G. *APMIS* **110**:554–558.
  31. **Horn, D. L., J. B. Zabriskie, R. Austrian, P. P. Cleary, J. J. Ferretti, V. A. Fischetti, E. Gotschlich, E. L. Kaplan, M. McCarty, S. M. Opal, R. B. Roberts, A. Tomasz, and Y. Wachtfogel.** 1998. Why have group A streptococci remained susceptible to penicillin? Report on a symposium. *Clin. Infect. Dis.* **26**:1341–1345.
  32. **Jensen, L. B., A. M. Hammerum, and F. M. Aarestrup.** 2000. Linkage of *vat(E)* and *erm(B)* in streptogramin-resistant *Enterococcus faecium* isolates from Europe. *Antimicrob. Agents Chemother.* **44**:2231–2232.
  33. **Kalia, A., M. C. Enright, B. G. Spratt, and D. E. Bessen.** 2001. Directional gene movement from human-pathogenic to commensal-like streptococci. *Infect. Immun.* **69**:4858–4869.
  34. **Laupland, K. B., T. Ross, D. L. Church, and D. B. Gregson.** 2006. Population-based surveillance of invasive pyogenic streptococcal infection in a large Canadian region. *Clin. Microbiol. Infect.* **12**:224–230.
  35. **Lawrence, J. G.** 1997. Selfish operons and speciation by gene transfer. *Trends Microbiol.* **5**:355–359.
  36. **Liu, L. C., J. C. Tsai, P. R. Hsueh, S. P. Tseng, W. C. Hung, H. J. Chen, and L. J. Teng.** 2008. Identification of *tet(S)* gene area in tetracycline-resistant *Streptococcus dysgalactiae* subsp. *equisimilis* clinical isolates. *J. Antimicrob. Chemother.* **61**:453–455.
  37. **McMillan, D. J., R. Geffers, J. Buer, B. J. Vlamincx, K. S. Sriprakash, and G. S. Chhatwal.** 2007. Variations in the distribution of genes encoding virulence and extracellular proteins in group A streptococcus are largely restricted to 11 genomic loci. *Microbes Infect.* **9**:259–270.
  38. **O'Connor, L., A. Coffey, C. Daly, and G. F. Fitzgerald.** 1996. AbiG, a genotypically novel abortive infection mechanism encoded by plasmid pCI750 of *Lactococcus lactis* subsp. *cremoris* UC653. *Appl. Environ. Microbiol.* **62**:3075–3082.
  39. **Paulsen, I. T., L. Banerjee, G. S. Myers, K. E. Nelson, R. Seshadri, T. D. Read, D. E. Fouts, J. A. Eisen, S. R. Gill, J. F. Heidelberg, H. Tettelin, R. J. Dodson, L. Umayam, L. Brinkac, M. Beanan, S. Daugherty, R. T. DeBoy, S. Durkin, J. Kolonay, R. Madupu, W. Nelson, J. Vamathevan, B. Tran, J. Upton, T. Hansen, J. Shetty, H. Khouri, T. Utterback, D. Radune, K. A. Ketchum, B. A. Dougherty, and C. M. Fraser.** 2003. Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. *Science* **299**:2071–2074.
  40. **Pavlovic, G., V. Burrus, B. Gintz, B. Decaris, and G. Guedon.** 2004. Evolution of genomic islands by deletion and tandem accretion by site-specific recombination: ICESt1-related elements from *Streptococcus thermophilus*. *Microbiology* **150**:759–774.
  41. **Perez-Casal, J., J. A. Price, E. Maguin, and J. R. Scott.** 1993. An M protein with a single C repeat prevents phagocytosis of *Streptococcus pyogenes*: use of a temperature-sensitive shuttle vector to deliver homologous sequences to the chromosome of *S. pyogenes*. *Mol. Microbiol.* **8**:809–819.
  42. **Raze, D., O. Dardenne, S. Hallut, M. Martinez-Bueno, J. Coyette, and J. M. Ghuyssen.** 1998. The gene encoding the low-affinity penicillin-binding protein 3r in *Enterococcus hirae* S185R is borne on a plasmid carrying other antibiotic resistance determinants. *Antimicrob. Agents Chemother.* **42**:534–539.
  43. **Rogers, Y. C., A. C. Munk, L. J. Meincke, and C. S. Han.** 2005. Closing bacterial genomic sequence gaps with adaptor-PCR. *BioTechniques* **39**:31–32, 34.
  44. **Rosen, B. P.** 1999. Families of arsenic transporters. *Trends Microbiol.* **7**:207–212.
  45. **Sitkiewicz, I., M. J. Nagiec, P. Sumbly, S. D. Butler, C. Cywes-Bentley, and J. M. Musser.** 2006. Emergence of a bacterial clone with enhanced virulence by acquisition of a phage encoding a secreted phospholipase A2. *Proc. Natl. Acad. Sci. USA* **103**:16009–16014.
  46. **Smith, M. D., and W. R. Guild.** 1980. Improved method for conjugative transfer by filter mating of *Streptococcus pneumoniae*. *J. Bacteriol.* **144**:457–459.
  47. **Stevens, D. L., M. H. Tanner, J. Winship, R. Swartz, K. M. Ries, P. M. Schlievert, and E. Kaplan.** 1989. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *N. Engl. J. Med.* **321**:1–7.
  48. **Sumbly, P., S. F. Porcella, A. G. Madrigal, K. D. Barbian, K. Virtaneva, S. M. Ricklefs, D. E. Sturdevant, M. R. Graham, J. Vuopio-Varkila, N. P. Hoe, and J. M. Musser.** 2005. Evolutionary origin and emergence of a highly successful clone of serotype M1 group A *Streptococcus* involved multiple horizontal gene transfer events. *J. Infect. Dis.* **192**:771–782.
  49. **Sylvetsky, N., D. Raveh, Y. Schlesinger, B. Rudensky, and A. M. Yinnon.** 2002. Bacteremia due to beta-hemolytic *Streptococcus* group G: increasing incidence and clinical characteristics of patients. *Am. J. Med.* **112**:622–626.
  50. **Tanous, C., E. Chambellon, and M. Yvon.** 2007. Sequence analysis of the mobilizable lactococcal plasmid pGdh442 encoding glutamate dehydrogenase activity. *Microbiology* **153**:1664–1675.
  51. **Tettelin, H., V. Masignani, M. J. Cieslewicz, C. Donati, D. Medini, N. L. Ward, S. V. Angiuoli, J. Crabtree, A. L. Jones, A. S. Durkin, R. T. DeBoy, T. M. Davidsen, M. Mora, M. Scarselli, I. Margarit y Ros, J. D. Peterson, C. R. Hauser, J. P. Sundaram, W. C. Nelson, R. Madupu, L. M. Brinkac, R. J. Dodson, M. J. Rosovitz, S. A. Sullivan, S. C. Daugherty, D. H. Haft, J. Selengut, M. L. Gwinn, L. Zhou, N. Zafar, H. Khouri, D. Radune, G. Dimitrov, K. Watkins, K. J. O'Connor, S. Smith, T. R. Utterback, O. White, C. E. Rubens, G. Grandi, L. C. Madoff, D. L. Kasper, J. L. Telford, M. R. Wessels, R. Rappuoli, and C. M. Fraser.** 2005. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial “pan-genome”. *Proc. Natl. Acad. Sci. USA* **102**:13950–13955.
  52. **Tettelin, H., V. Masignani, M. J. Cieslewicz, J. A. Eisen, S. Peterson, M. R. Wessels, I. T. Paulsen, K. E. Nelson, I. Margarit, T. D. Read, L. C. Madoff, A. M. Wolf, M. J. Beanan, L. M. Brinkac, S. C. Daugherty, R. T. DeBoy, A. S. Durkin, J. F. Kolonay, R. Madupu, M. R. Lewis, D. Radune, N. B. Fedorova, D. Scanlan, H. Khouri, S. Mulligan, H. A. Carty, R. T. Cline, S. E. Van Aken, J. Gill, M. Scarselli, M. Mora, E. T. Iacobini, C. Brettoni, G. Galli, M. Mariani, F. Vegni, D. Maione, D. Rinaudo, R. Rappuoli, J. L. Telford, D. L. Kasper, G. Grandi, and C. M. Fraser.** 2002. Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V *Streptococcus agalactiae*. *Proc. Natl. Acad. Sci. USA* **99**:12391–12396.
  53. **Van Domselaar, G. H., P. Stothard, S. Shrivastava, J. A. Cruz, A. Guo, X. Dong, P. Lu, D. Szafron, R. Greiner, and D. S. Wishart.** 2005. BASys: a web server for automated bacterial genome annotation. *Nucleic Acids Res.* **33**:W455–W459.
  54. **Walker, M. J., A. Hollands, M. L. Sanderson-Smith, J. N. Cole, J. K. Kirk, A. Henningham, J. D. McArthur, K. Dinkla, R. K. Aziz, R. G. Kansal, A. J. Simpson, J. T. Buchanan, G. S. Chhatwal, M. Kotb, and V. Nizet.** 2007. DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. *Nat. Med.* **13**:981–985.
  55. **Werner, G., B. Hildebrandt, I. Klare, and W. Witte.** 2000. Linkage of determinants for streptogramin A, macrolide-lincosamide-streptogramin B, and chloramphenicol resistance on a conjugative plasmid in *Enterococcus faecium* and dissemination of this cluster among streptogramin-resistant enterococci. *Int. J. Med. Microbiol.* **290**:543–548.
  56. **Williams, G. S.** 2003. Group C and G streptococci infections: emerging challenges. *Clin. Lab Sci.* **16**:209–213.
  57. **Yang, H. C., J. Cheng, T. M. Finan, B. P. Rosen, and H. Bhattacharjee.** 2005. Novel pathway for arsenic detoxification in the legume symbiont *Sinorhizobium meliloti*. *J. Bacteriol.* **187**:6991–6997.
  58. **Zhang, S., N. M. Green, I. Sitkiewicz, R. B. Lefebvre, and J. M. Musser.** 2006. Identification and characterization of an antigen I/II family protein produced by group A *Streptococcus*. *Infect. Immun.* **74**:4200–4213.