Comparative Genomic Hybridizations of Hospital and Community-associated MRSA in Canada

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ABSTRACT

Background: Surveillance of Canadian MRSA isolates collected since 1995 has identified 10 epidemic strains (CMRSA-1 to 10) and various sporadic strains (##S-####) currently present in hospitals and the community. In order to better understand the genetic content of these strains, we have used microarrays to compare their genomes to that of the fully characterized MRSA strain COL.

Methods: Genomic DNA was fluorescently labelled using random primed labelling. DNA from each of the strains, along with COL was hybridised to arrays containing 2740 of the open reading frames (ORFs) from COL. Data was processed with the ArrayPro, GACK and GeneMaths computer programs. Macrorestriction digest patterns were generated with pulsed-field gel electrophoresis (PFGE) using SmaI and analyzed using Bionumerics software. Multi-locus sequence typing (MLST) was preformed as described at http://www.mlst.net.

Results: Results indicate that there are currently no ORFs whose presence or absence can predict whether a strain is epidemic, sporadic, nosocomial or community acquired. Analysis of toxin content showed that CMRSA-1, 2, 4, and 8 as well as 02S-0250 carry fewer toxins than CMRSA-3, 5, 6, 7, 9, 10 and the 3 other sporadic strains. Comparison of array data with PFGE and MLST create slightly different phylogenies. Though many of the strains have similar genome sizes, some share less genetic content in common with COL when compared to others. For example, both CMRSA 1 and 3 have genomes of approximately 2400 kb but CMRSA-1 only shares 81% of its genetic content with COL while CMRSA-3 shares 92%. The same pattern exists in the sporadic strains, suggesting there may be significant genetic content not represented on the array but present in some Canadian MRSA. This may also account for differences when compared with molecular typing methods.

Conclusions: From this analysis, there are no ORFs whose presence or absence can differentiate between the various types of MRSA strains. PFGE, MLST and arrays create different phylogenetic patterns that may be due to unique genetic content of experimental strains not present on the arrays.

INTRODUCTION

Methicillin resistance in *Staphylococcus aureus* emerged more than 40 years ago in the United Kingdom only briefly after the introduction of methicillin (1). In the following decades methicillin-resistant *S. aureus* (MRSA) strains have been identified and become epidemic in hospitals worldwide, and are currently among the most prevalent hospital acquired infections in the United States (4) with case numbers on the rise in Canada (7).

Starting in 1995, hospitals belonging to the Canadian Nosocomial Infection Surveillance Program have submitted MRSA strains to our laboratory for the purpose of surveillance (7). These strains are then analyzed using pulsed-field gel electrophoresis (PFGE) in order to elucidate their relatedness to one another. In Canada, there are currently ten epidemic groups of MRSA. An epidemic strain is one which is considered to be clinically significant and is isolated from five or more hospital sites or from three or more geographical regions across Canada (6). These strains have been given the annotation CMRSA for Canadian MRSA. There are also various other strains which are considered non-epidemic or sporadic. Each of the ten epidemic groups contains strains which were shown to be closely related by PFGE banding patterns (8, unpublished data). Each CMRSA group has unique traits. CMRSA 1and 2 are the most prevalent strains, CMRSA 7 and 10 are more likely to be community acquired and are associated with outbreaks in the community in the United States, and CMRSA 5 and 6 are associated with a single hospital site (8). Through surveillance, we have also identified strains whose PFGE patterns have only occurred once and are distantly related to the epidemic strains. These have been denoted sporadic strains. In this study, we have used a comparative genomic hybridization approach to compare the genetic content, as well as the relatedness of these ten epidemic strains and four sporadic MRSA strains found in Canada. We have also been able to compare the strains associated with the community to those more commonly associated with the hospital.

MATERIALS & METHODS

PCR products (primers purchased from Sigma-Genosys, U.K.) and 70 base pair oligomers representing the complete open reading frame (ORF) set from the MRSA strain COL were spotted in duplicate onto Corning UltraGAPS (Corning Inc., U.S.A.) slides in a solution of 50% Dimethyl Sulfoxide by the DNA core facility at the Canadian National Microbiology Laboratory, Winnipeg, MB. DNA was extracted from all strains using methods previously described (5) and randomly fragmented by a two minute thirty second sonication. DNA fragments were then labeled with using the BioPrime Array CGH kit (Invitrogen, U.S.A.) and Cy3 or Cy5 labeled dCTP (Amersham Biosciences, U.K.). Labeled probes were hybridized to the slides and the slides washed according the manufacturer's directions for the UltraGAPS slides. Slides were hybridized with both a sample strain as well as the COL strain, each labeled with different dyes. For each MRSA strain, hybridizations were performed in triplicate, with one of the hybridizations being a dye switch experiment.

After washing, slides were scanned using the Virtek ChipReader (Bio-Rad, U.S.A.) and subsequently analyzed using the Array-Pro Analyzer 4.5 software package (Media Cybernetics Inc., U.S.A.), the GACK program (3) and the Gene Maths Software package (Applied Maths, Belgium).

Pulsed-field gel electrophoresis (PFGE) was performed as previously described by the Centers for Disease Control and Prevention (2). PFGE dendrograms were created using the BioNumerics software package (Applied Maths, Belgium). Multilocus sequence typing was performed according to the protocol available at www.MLST.net.

Canada

RESULTS

Table 1: Presence or absence/divergence of adhsins in Canadian epidemic and sporadic MRSA strains

NAME	GENE	DESCRIPTION	CMRSA1	CMRSA2	CMRSA3	CMRSA4	CMRSA5	CMRSA6	CMRSA7	CMRSA8	CMRSA9	CMRSA10	98S-1344	99S-1088	00S-1635	02S-0250
SA0095	-	Protein A precursor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0608	sdrC	sdrC protein	-	+	+	-	+	+	+	+	+	+	+	n/a	-	+
SA0609	sdrD	sdrD protein	n/a	+	+	-	+	+	+	+	+	+	+	+	+	+
SA0610	sdrE	sdrE protein	+	+	+	+	+	+	+	+	+	+	+	+	-	+
SA0858	ssp	secretory extracellular matrix and plasma binding protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1168	fib	fibrinogen-binding protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1169	fib	fibrinogen-binding protein precursor related protein	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA1220	-	fibronectin-binding protein A-related	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1522	ebpS	elastin binding protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2509	fnbB	fibronectin binding protein B	+	+	+	-	+	+	+	-	+	+	+	+	+	+
SA2511	fnbA	fibronectin-binding protein A	+	-	+	-	+	+	-	-	+	+	+	+	-	-
SA2652	clfB	clumping factor B	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2688	icaR	intercellular adhesion regulator	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2689	icaA	icaA protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2690	icaD	icaD protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2691	icaB	icaB protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2692	icaC	icaC protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2: Presence or absence/divergence of exoenzymes in Canadian epidemic and sporadic MRSA strains

NAME	GENE	DESCRIPTION	CMRSA1	CMRSA2	CMRSA3	CMRSA4	CMRSA5	CMRSA6	CMRSA7	CMRSA8	CMRSA9	CMRSA10	98S-1344	99S-1088	00S-1635	02S-0250
SA0024	-	5-nucleotidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0209	coa	coagulase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0317	geh	"lipase precursor, interruption"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0712	-	lipase/esterase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0857	-	"coagulase, putative"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0860	nuc	thermonuclease precursor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1028	-	serine protease	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1056	-	staphopain	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1057	-	glutamyl endopeptidase precursor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1357	-	staphylococcal nuclease family protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1864	-	serine protease SplF	-	+	+	+	+	+	+	-	+	+	-	-	-	+
SA1865	-	serine protease SplE	-	-	+	+	+	+	-	-	+	+	-	-	-	-
SA1866	-	serine protease SplD	-	+	+	+	+	+	-	-	+	+	-	-	-	+
SA1867	-	serine protease SplC	-	+	+	n/a	+	+	+	-	+	+	+	-	-	+
SA1868	-	serine protease SplB	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA1869	-	serine protease SplA	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA1970	-	staphopain	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2003	-	phospholipase C precursor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2194	hysA	hyaluronate lyase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2549	-	"esterase, putative"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2659	aur	aureolysin	n/a	+	+	-	+	n/a	+	+	+	+	+	+	+	+
SA2694	lip	lipase	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3: Presence or absence/divergence of toxins in Canadian epidemic and sporadic MRSA strains

NAME	GENE	DESCRIPTION	CMRSA1	CMRSA2	CMRSA3	CMRSA4	CMRSA5	CMRSA6	CMRSA7	CMRSA8	CMRSA9	CMRSA10	98S-1344	99S-1088	00S-1635	02S-0250
SA0265	-	Diarrheal toxin-like protein	n/a	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0468	-	exotoxin 3	n/a	+	+	-	+	+	+	-	+	+	+	+	-	+
SA0472	-	exotoxin 2	-	+	+	-	+	+	+	-	+	+	+	+	+	+
SA0473	-	exotoxin 5	-	+	+	-	+	+	+	+	+	+	+	+	+	+
SA0478	-	exotoxin 3	-	-	+	-	+	+	-	-	+	+	+	+	-	-
SA0762	-	"hemolysin, putative"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA0886	ent	staphylococcal enterotoxin	-	-	+	-	+	+	+	-	-	+	+	+	+	-
SA0887	sei	staphylococcal enterotoxin type I	-	-	+	-	+	+	+	-	+	+	-	+	+	-
SA0907	seb	staphylococcal enterotoxin B	-	-	-	-	+	-	-	-	+	-	-	+	-	-
SA1178	-	"exotoxin 1, putative"	-	+	+	-	+	+	+	-	+	+	+	+	+	+
SA1179	-	"exotoxin 4, putative"	-	+	+	-	+	+	+	-	+	+	+	+	+	+
SA1180	-	"exotoxin 3, putative"	-	+	+	-	+	+	+	+	+	+	+	+	+	+
SA1657	-	enterotoxin family protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA1880	lukD	leukotoxin LukD	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA1881	lukS	synergohymenotropic toxin lukS	-	+	+	-	+	+	+	-	+	+	+	-	+	+
SA2004	lukF	"leukocidin precursor, F subunit, putative"	+	+	+	-	+	+	+	+	+	+	+	+	+	+
SA2006	lukM	leukotoxin LukM	-	+	+	-	+	+	+	+	+	+	+	+	+	+
SA2022	hld	delta-hemolysin	+	+	+	+	+	+	+	+	+	+	n/a	+	n/a	+
SA2160	-	"hemolysin, putative"	+	+	+	+	+	+	+	-	+	+	+	+	+	+
SA2419	-	"gamma-hemolysin, component A"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2421	-	"leukocidin precursor, S subunit"	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SA2422	-	"leukocidin precursor, F subunit"	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: (+) present, (-) absent, (n/a) no data

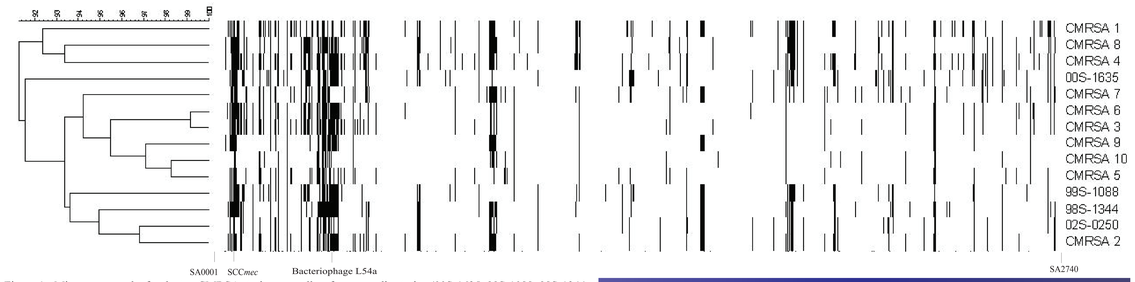

Figure 1: Microarray results for the ten CMRSA strains, as well as four sporadic strains (00S-1635, 99S-1088, 98S-1344, 02S-0250) . White represents the presence of an open reading frame, black represents its absence or divergence.

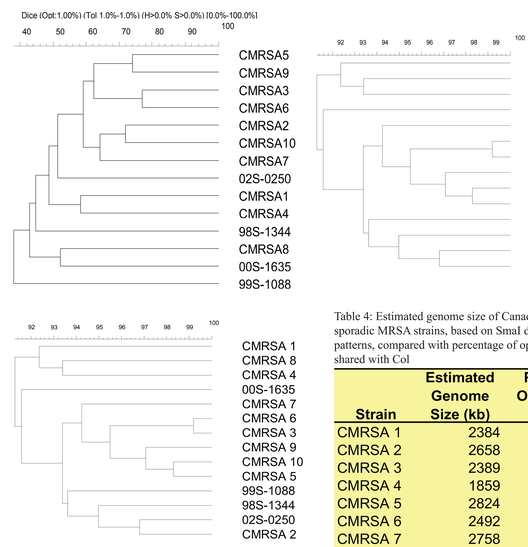

Figure 2. Comparison of phylogenetic trees created from microarray data (A), and pulsed-field gel electrophoresis data (B). Scale for (A) represents percent similarity calculated on the basis of the Euclidian distance coefficient, and scale for (B) represents percent similarity calculated on the basis of the Dice coefficient. Cluster analysis performed using unweighted pair group method, using arithmetic averages (UPGMA).

Table 4: Estimated genome size of Canadian epidemic and sporadic MRSA strains, based on SmaI digestions banding patterns, compared with percentage of open reading frames shared with Col

Strain	Estimated Genome Size (kb)	Percentage ORFs Shared with Col
CMRSA 1	2384	90.21%
CMRSA 2	2658	92.46%
CMRSA 3	2389	95.44%
CMRSA 4	1859	89.56%
CMRSA 5	2824	97.99%
CMRSA 6	2492	93.92%
CMRSA 7	2758	93.46%
CMRSA 8	1973	89.56%
CMRSA 9	2629	95.82%
CMRSA 10	2659	97.83%
98S-1344	2576	91.37%
99S-1088	2348	92.32%
00S-1635	2278	90.03%
02S-0250	2804	93.20%

CONCLUSIONS

While similarities and differences exist between all Canadian MRSA strains, none are indicative of the epidemic or sporadic nature of the strains, nor can they distinguish community associated strains from nosocomial strains

CMRSA 1, 4 and 8, along with 99S-1088 carry fewer toxin and virulence genes than the other strains studied, but again there is no notable difference between the toxin and virulence genes carried by community strains and nosocomial strains.

Comparison of estimated genome size and percentage of genes present on the array indicates that some strains may have more genetic content not represented on the array than others.

Phylogenetic analysis by arrays, MLST and PFGE create very similar dendrograms, with some minor differences. Some of these differences are most likely due to the genetic content not represented on the array. This suggests that comparative genomic hybridization experiments may not be an appropriate tool for determining lineages of strains.

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Acknowledgements: We would like to thank Shari Tyson and Claude Ouellette of the DNA Core Facility (National Microbiology Laboratory, Winnipeg, Canada) for their technical assistance and guidance in developing and printing our microarrays.

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