How to make use of the laboratory in the control of MRSA

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Historical perspective

- 1960's: MRSA appeared
  - Usually as nosocomial infections
  - Uncommon in community
- 1993: First report of emerging CA-MRSA strains (Western Australia)
- CA-MRSA infection notifiable in Hong Kong since 5 January 2007

Definition of "methicillin-resistance"

- *mecA* gene mediated:
  - Resistant to cefoxitin
  - Resistant to oxacillin with high MIC
- Hyperproduction of beta-lactamase:
  - Susceptible to cefoxitin
  - Resistant to oxacillin with low MIC (≥4 μg/ml)
- Both to be reported as MRSA according to CLSI
- Former with greater infection control and management significance

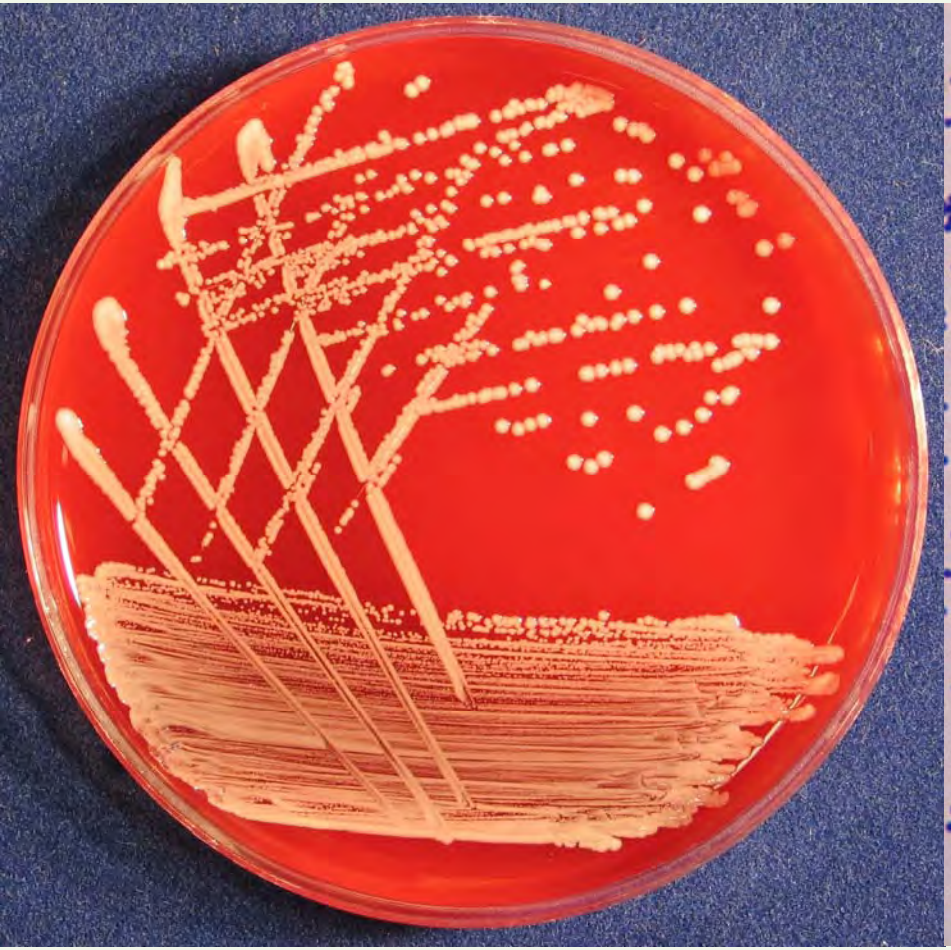
Role of laboratory

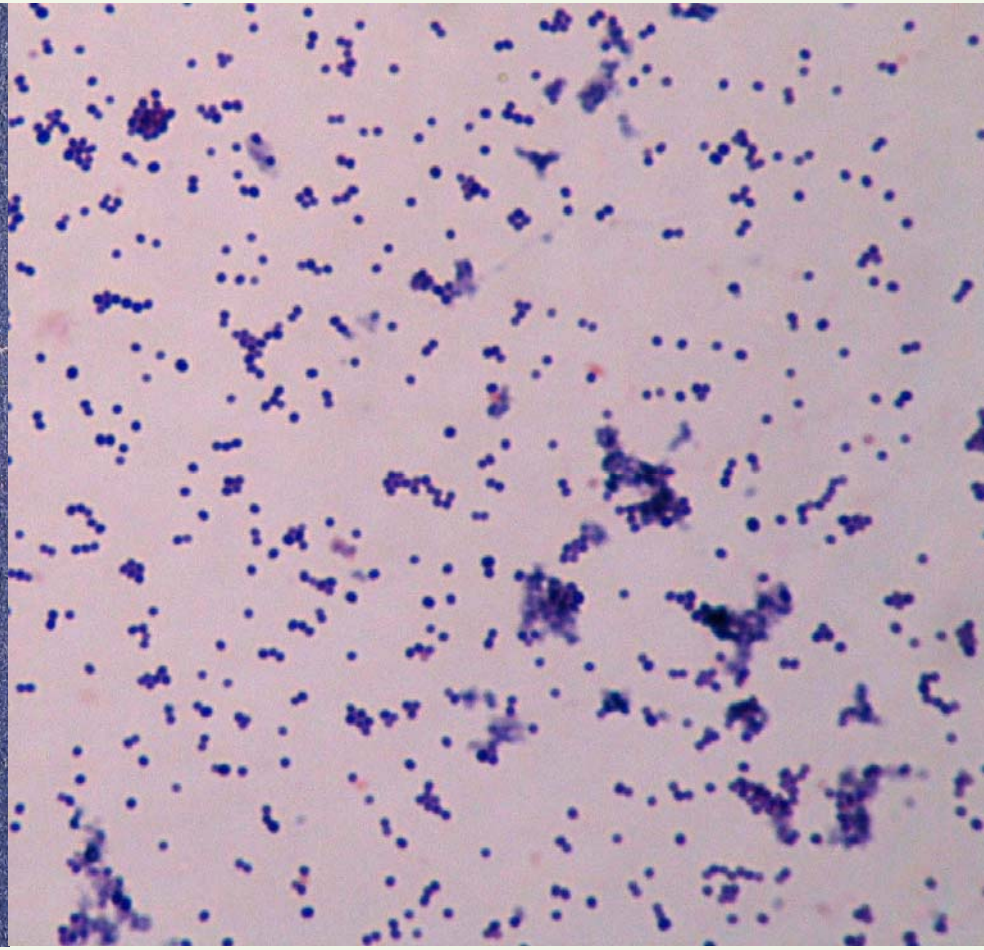
- Detection
- Antimicrobial susceptibility
- Typing

Detection of MRSA

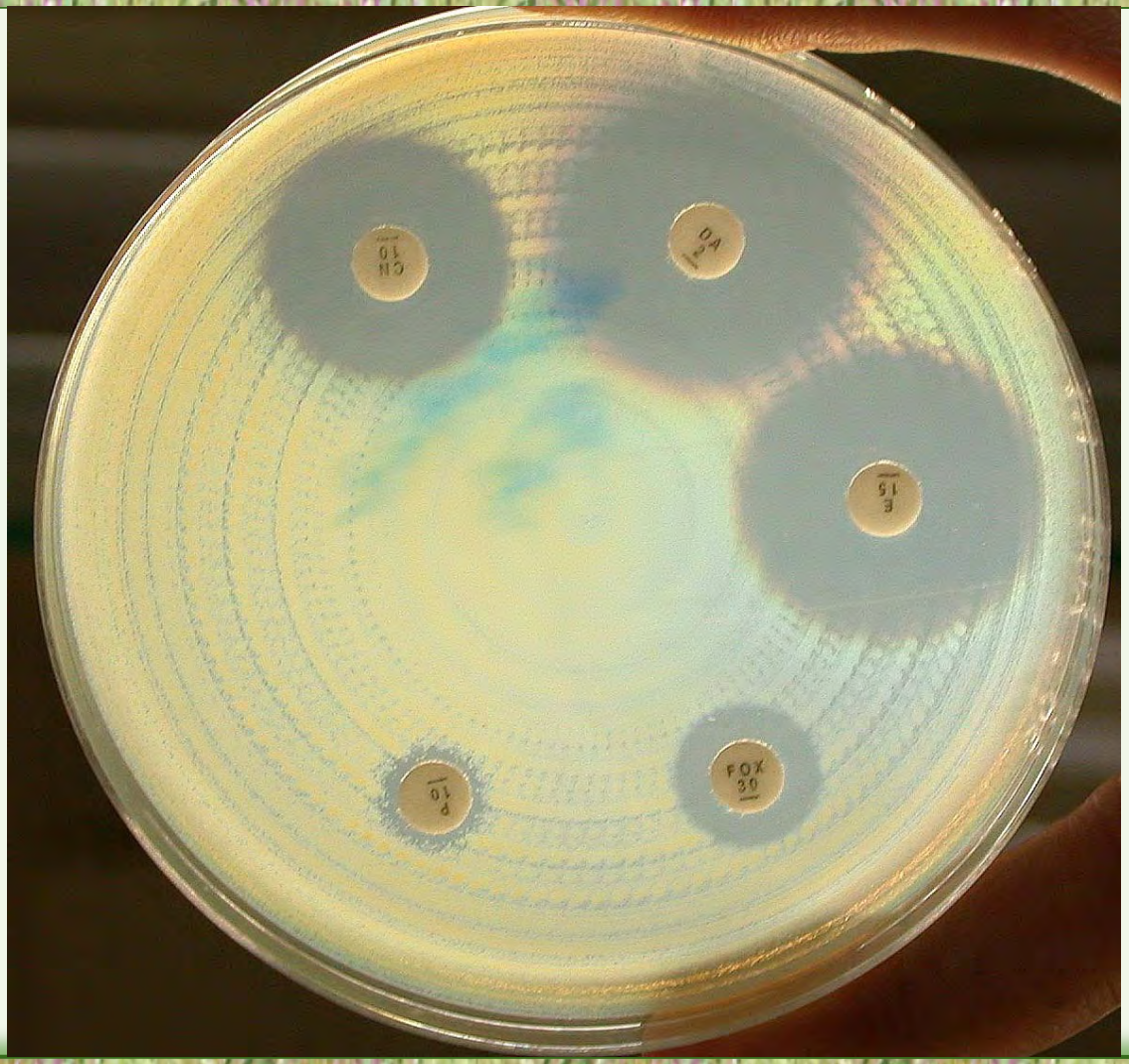
- From clinical specimens
  - Isolation by culture
    - Identification (phenotypic)
    - Resistance to cefoxitin / oxacillin
  - Direct molecular detection
    - e.g. *nuc* and *mecA* gene detection
    - In-house or commercial platforms
    - Also for screening

Detection of MRSA





Detection of MRSA



Detection of MRSA

- Screening
  - Isolation by culture
    - Enrichment to increase sensitivity (e.g. nutrient broth/6.5% NaCl overnight)
    - Inoculation to differential/selective medium (e.g. commercial chromogenic agar with cefoxitin/oxacillin)
  - Molecular detection

Detection of MRSA

Antimicrobial susceptibility

- For clinical management (patient treatment, decolonization of carriers)
- For epidemiological information (e.g. CA-MRSA reportedly relatively sensitive to most antibiotics, compared to HA-MRSA)
  - Multi-resistant CA-MRSA phenotypes not uncommonly encountered in Hong Kong
- For typing purpose: Antibiogram

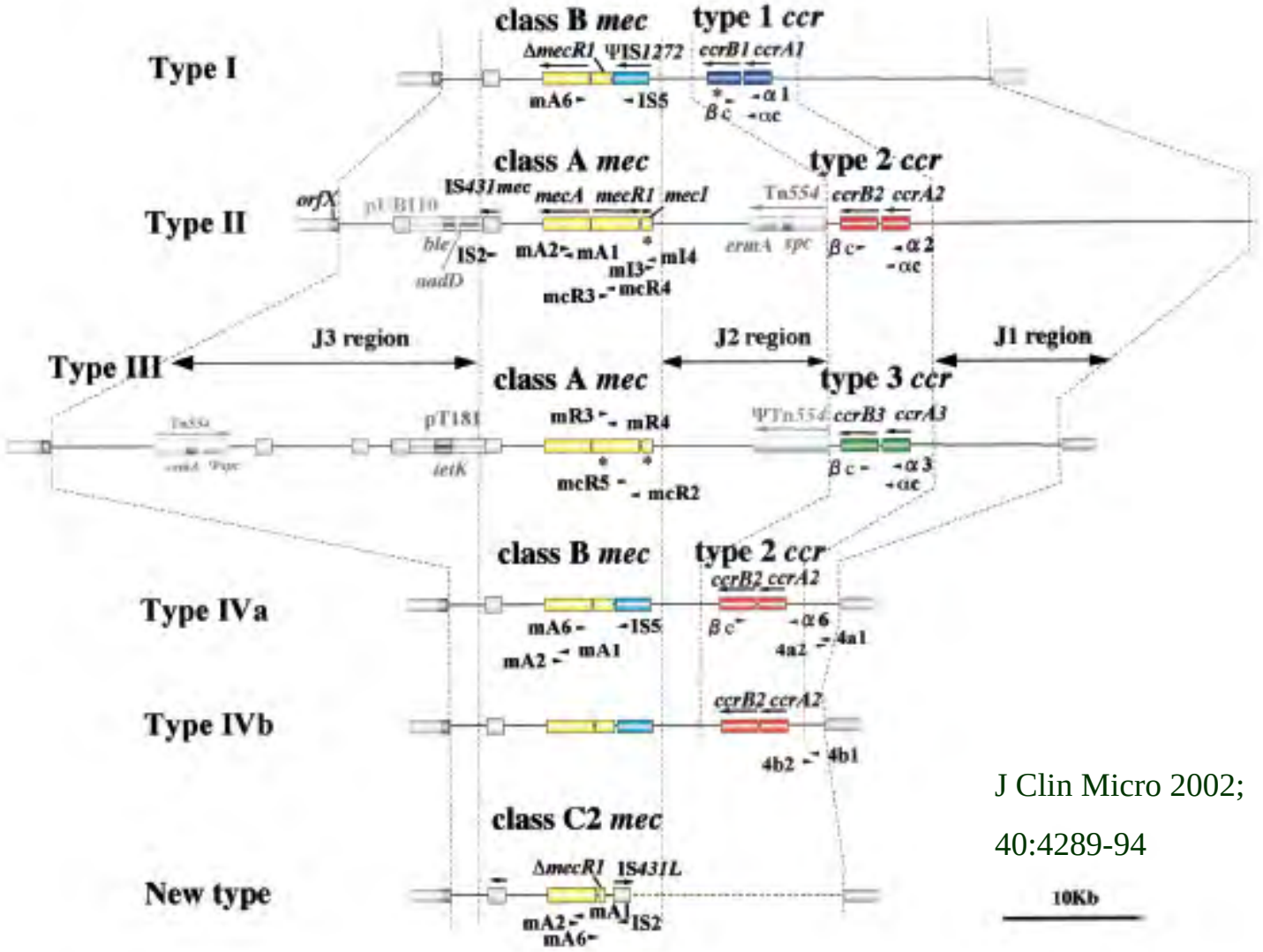
Typing of S. aureus

- SCC*mec* typing
- Multi-locus sequence typing (MLST)
- Pulsed-field gel electrophoresis (PFGE)
- *spa* typing

Staphylococcal cassette chromosome

- SCC: A unique family of mobile genetic elements found on the chromosomes of *Staphylococcus spp.*
- If carries *mecA* gene, then called SCC*mec*
- SCC*mec* typing:
  - *mec* gene complex
  - *ccr* (chromosomal cassette recombinase) gene complex

SCCmec typing



J Clin Micro 2002;

40:4289-94

*SCC*mec *typing*

SCC*mec* type	*mec* (class)	*ccr* (type)
I	B	1
II	A	2
III	A	3
IV	B	2
V	C2	5

SCCmec typing

- SCC*mec* types I to III
  - Found in hospital strains (Type I strains discovered in 1960's)
- SCC*mec* types IV and V
  - Associated with CA-MRSA strains
  - SCC*mec* type IV found in MW2 strain (2002)
  - SCC*mec* type V first described in 2004 (strain reported in 1999 from Western Australia)

SCCmec typing

- Crude typing method
- Part of notifiable disease case definition for CA-MRSA in Hong Kong

MLST

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2000, p. 1008–1015 Vol. 38, No. 3
0095-1137/00/$04.00+0

Multilocus Sequence Typing for Characterization of Methicillin-Resistant and Methicillin-Susceptible Clones of *Staphylococcus aureus*

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Received 22 September 1999/Accepted 15 December 1999

A multilocus sequence typing (MLST) scheme has been developed for *Staphylococcus aureus*. The sequences of internal fragments of seven housekeeping genes were obtained for 155 *S. aureus* isolates from patients with community-acquired and hospital-acquired invasive disease in the Oxford, United Kingdom, area. Fifty-three different allelic profiles were identified, and 17 of these were represented by at least two isolates. The MLST scheme was highly discriminatory and was validated by showing that pairs of isolates with the same allelic profile produced very similar *Sma*I restriction fragment patterns by pulsed-field gel electrophoresis. All 22 isolates with the most prevalent allelic profile were methicillin-resistant *S. aureus* (MRSA) isolates and had allelic profiles identical to that of a reference strain of the epidemic MRSA clone 16 (EMRSA-16). Four MRSA isolates that were identical in allelic profile to the other major epidemic MRSA clone prevalent in British hospitals (clone EMRSA-15) were also identified. The majority of isolates (81%) were methicillin-susceptible *S. aureus* (MSSA) isolates, and seven MSSA clones included five or more isolates. Three of the MSSA clones included at least five isolates from patients with community-acquired invasive disease and may represent virulent clones with an increased ability to cause disease in otherwise healthy individuals. The most prevalent MSSA clone (17 isolates) was very closely related to EMRSA-16, and the success of the latter clone at causing disease in hospitals may be due to its emergence from a virulent MSSA clone that was already a major cause of invasive disease in both the community and hospital settings. MLST provides an unambiguous method for assigning MRSA and MSSA isolates to known clones or assigning them as novel clones via the Internet.

MLST

- Commonly used in population studies
- Typing method of medium discriminatory power
- Technically relatively simple and facilitates inter-laboratory comparison of results

PFGE

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2003, p. 5113–5120 Vol. 41, No. 11
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.11.5113–5120.2003

Pulsed-Field Gel Electrophoresis Typing of Oxacillin-Resistant *Staphylococcus aureus* Isolates from the United States: Establishing a National Database

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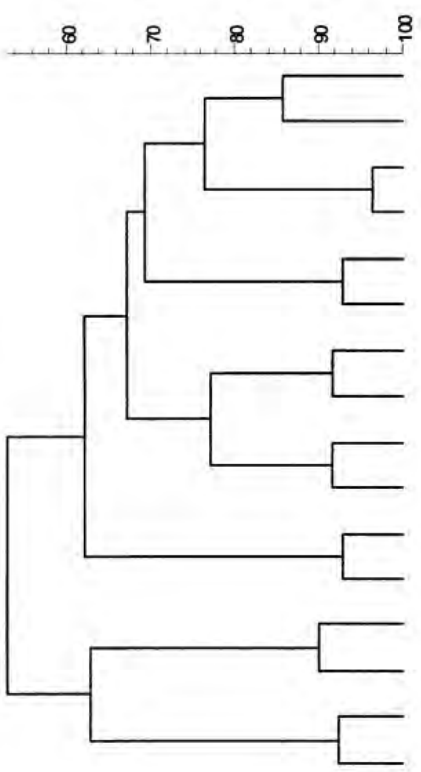
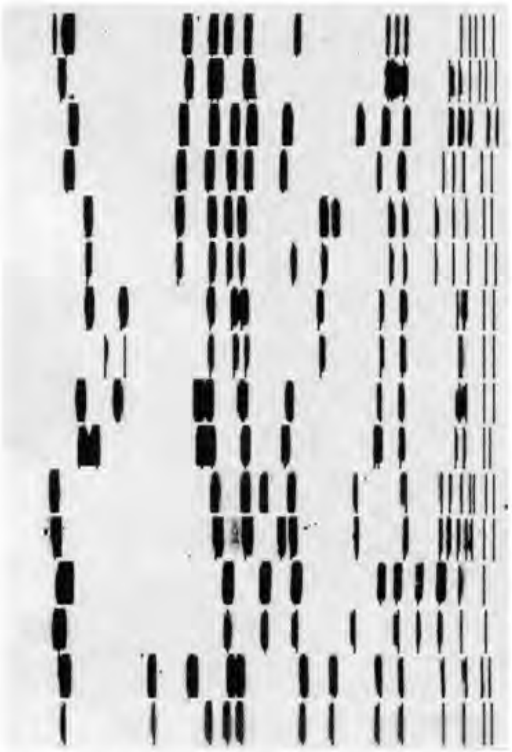
Received 2 June 2003/Returned for modification 10 July 2003/Accepted 22 August 2003

Oxacillin-resistant *Staphylococcus aureus* (ORSA) is a virulent pathogen responsible for both health care-associated and community onset disease. We used *Sma*I-digested genomic DNA separated by pulsed-field gel electrophoresis (PFGE) to characterize 957 *S. aureus* isolates and establish a database of PFGE patterns. In addition to PFGE patterns of U.S. strains, the database contains patterns of representative epidemic-type strains from the United Kingdom, Canada, and Australia; previously described ORSA clonal-type isolates; 13 vancomycin-intermediate *S. aureus* (VISA) isolates, and two high-level vancomycin-resistant, *vanA*-positive strains (VRSA). Among the isolates from the United States, we identified eight lineages, designated as pulsed-field types (PFTs) USA100 through USA800, seven of which included both ORSA and oxacillin-susceptible *S. aureus* isolates. With the exception of the PFT pairs USA100 and USA800, and USA300 and USA500, each of the PFTs had a unique multilocus sequence type and *spa* type motif. The USA100 PFT, previously designated as the New York/Tokyo clone, was the most common PFT in the database, representing 44% of the ORSA isolates. USA100 isolates were typically multiresistant and included all but one of the U.S. VISA strains and both VRSA isolates. Multiresistant ORSA isolates from the USA200, -500, and -600 PFTs have PFGE patterns similar to those of previously described epidemic strains from Europe and Australia. The USA300 and -400 PFTs contained community isolates resistant only to β-lactam drugs and erythromycin. Noticeably absent from the U.S. database were isolates with the previously described Brazilian and EMRSA15 PFGE patterns. These data suggest that there are a limited number of ORSA genotypes present in the United States.

PFGE

- 622 out of 667 MRSA strains clustered with 8 pulse-field types (80% similarity)





PFT	MLST
USA700	72
USA300	8
USA400	1
USA800	5
USA100	5
USA600	45
USA200	36
USA500	8

FIG. 1. Dendrogram of PFTs with type strain (most frequent pattern) and a variant strain. Also shown is the corresponding MLST for each PFT (18, 19, 20).

PFGE

- Versatile typing method
- High discriminatory power (need to take into account epidemiological correlation)
- Technically demanding and less straight-forward inter-laboratory comparison of results

spa typing

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1999, p. 3556–3563 Vol. 37, No. 11
0095-1137/99/$04.00+0

Evaluation of Protein A Gene Polymorphic Region DNA Sequencing for Typing of *Staphylococcus aureus* Strains

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Received 27 January 1999/Returned for modification 5 June 1999/Accepted 11 July 1999

Three hundred and twenty isolates of *Staphylococcus aureus* were typed by DNA sequence analysis of the X region of the protein A gene (*spa*). *spa* typing was compared to both phenotypic and molecular techniques for the ability to differentiate and categorize *S. aureus* strains into groups that correlate with epidemiological information. Two previously characterized study populations were examined. A collection of 59 isolates (F. C. Tenover, R. Arbeit, G. Archer, J. Biddle, S. Byrne, R. Goering, G. Hancock, G. A. Hébert, B. Hill, R. Hollis, W. R. Jarvis, B. Kreiswirth, W. Eisner, J. Maslow, L. K. McDougal, J. M. Miller, M. Mulligan, and M. A. Pfaller, J. Clin. Microbiol. 32:407–415, 1994) from the Centers for Disease Control and Prevention (CDC) was used to test for the ability to discriminate outbreak from epidemiologically unrelated strains. A separate collection of 261 isolates form a multicenter study (R. B. Roberts, A. de Lencastre, W. Eisner, E. P. Severina, B. Shopsin, B. N. Kreiswirth, and A. Tomasz, J. Infect. Dis. 178:164–171, 1998) of methicillin-resistant *S. aureus* in New York City (NYC) was used to compare the ability of *spa* typing to group strains along clonal lines to that of the combination of pulsed-field gel electrophoresis and Southern hybridization. In the 320 isolates studied, *spa* typing identified 24 distinct repeat types and 33 different strain types. *spa* typing distinguished 27 of 29 related strains and did not provide a unique fingerprint for 4 unrelated strains from the four outbreaks of the CDC collection. In the NYC collection, *spa* typing provided a clonal assignment for 185 of 195 strains within the five major groups previously described. *spa* sequencing appears to be a highly effective rapid typing tool for *S. aureus* that, despite some expense of specificity, has significant advantages in terms of speed, ease of use, ease of interpretation, and standardization among laboratories.

A study on PVL positive MRSA

- JMM 2008; 57: 1440
- 42 strains from 2006
- 5 MLST types
- 5 PFGE patterns (80% similarity cut-off)
- 11 *spa* types – 5 lineages
- Concordance of typing methods demonstrated

A study on PVL positive MRSA

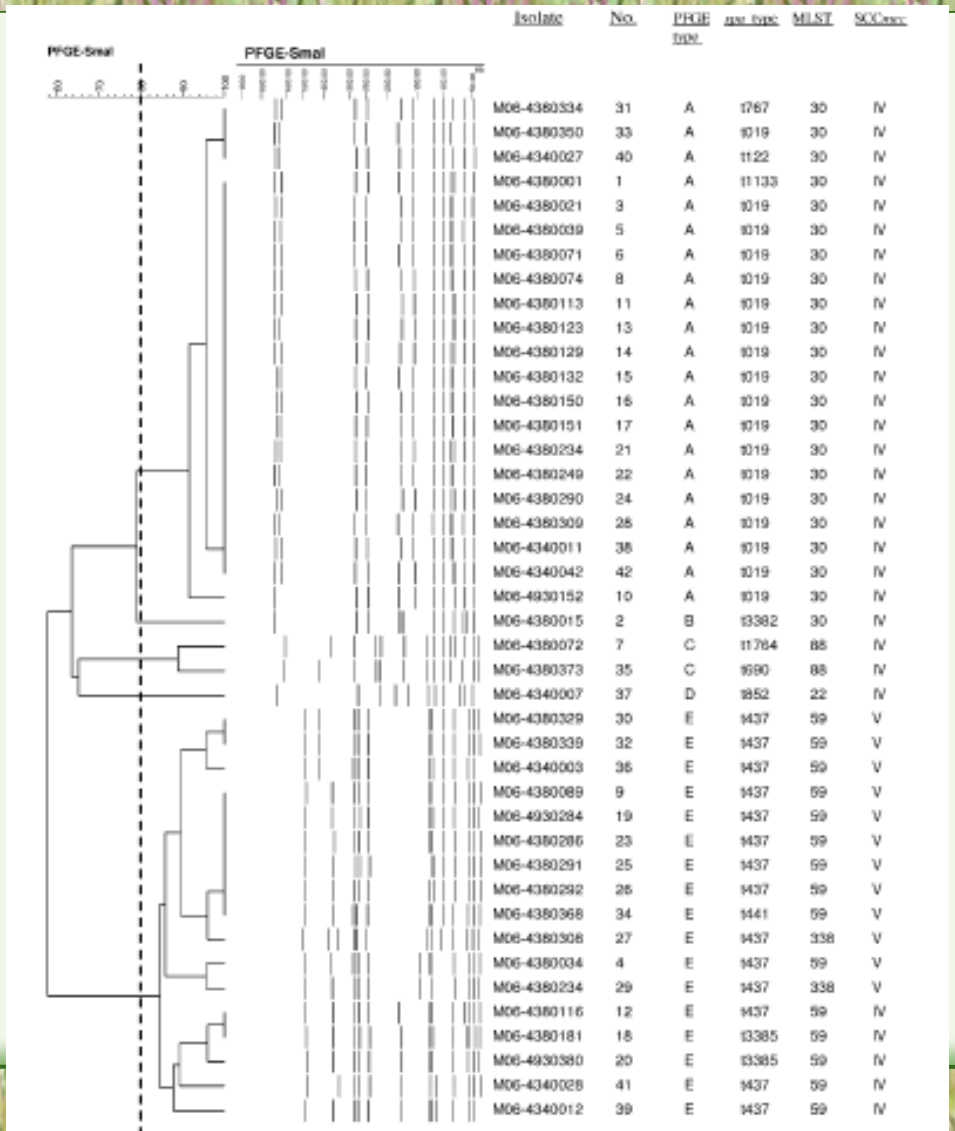
SCC*mec*	MLST			Ridom *spa* typing		Specimen type (no. of isolates)	Antibiotic resistance profile (no. of isolates)
	ST type	Allelic profile	No. of isolates (%)	*spa* type (no. of isolates)	*spa*-repeat		
IV	ST22	7-6-1-5-8-8-6	1 (2.4)	t852 (1)	r07r23r13r23r31r05r17r25r-17r25r16r28	Pus swab (1)	POF-GEM (1)
	ST30	2-2-2-2-6-3-2	22 (52.4)	t019 (18)	r08r16r02r16r02r25r17r24	Abscess (8), pus swab (5), wound swab (4), tissue (3), blood culture (1), joint aspirate (1)	POF (17), POF-CIP (3), POF-TET (1), POF-ERY-CLI-SXT-TET-RIF (1)
				t122 (1)	r08r16r02r16r02r25r17r24r24		
				t767 (1)	r08r16r02r16r02r16r02r25r17r24		
				t1133(1)	r08r16r02r16r02r25r02r25r17r24		
				Novel* (1)	r09r02r16r02r25r17r24		
	ST59	19-23-15-2-19-20-15	5 (11.9)	t437 (3)	r04r20r17r20r17r25r34	Abscess (1), pus swab (2), wound swab (2)	POF-ERY-CLI (1), POF-TET (1), POF-ERY-CLI-SXT (1), POF-ERY-CLI-TET (2)
				Novel† (2)	r04r20r17r20r16r34		
	ST88	22-1-14-23-12-4-31	2 (4.8)	t690 (1)	r07r12r21r17r13r13r34r34r34r33-r34	Pus swab (1), wound swab (1)	POF (1), POF-ERY-CLI-TET-CLI (1)
				t1764 (1)	r07r12r21r17r13r13r34r34r34r34-r33r13		
V	ST59	19-23-15-2-19-20-15	10 (23.8)	t437 (9)	r04r20r17r20r17r25r34	Abscess (2), pus swab (3), wound swab (2), blood culture (2), eye swab (1)	POF-ERY-CLI (2), POF-ERY-CLI-CHL (3), POF-ERY-CLI-TET-CHL (5)
				t441 (1)	r04r20r17r25r34		
	ST338	19-23-15-48-19-20-15	2 (4.8)	t437 (2)	r04r20r17r20r17r25r34	Wound swab (2)	POF-ERY-CLI-TET (1), POF-ERY-CLI-TET-CHL (1)
	Total		42				

CHL, Chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEM, gentamicin; POF, penicillin/oxacillin/cefoxitin; RIF, rifampicin; SXT, trimethoprim–sulfamethoxazole; TET, tetracycline.

*Registered as t3382.

†Registered as t3385.

A study on PVL positive MRSA

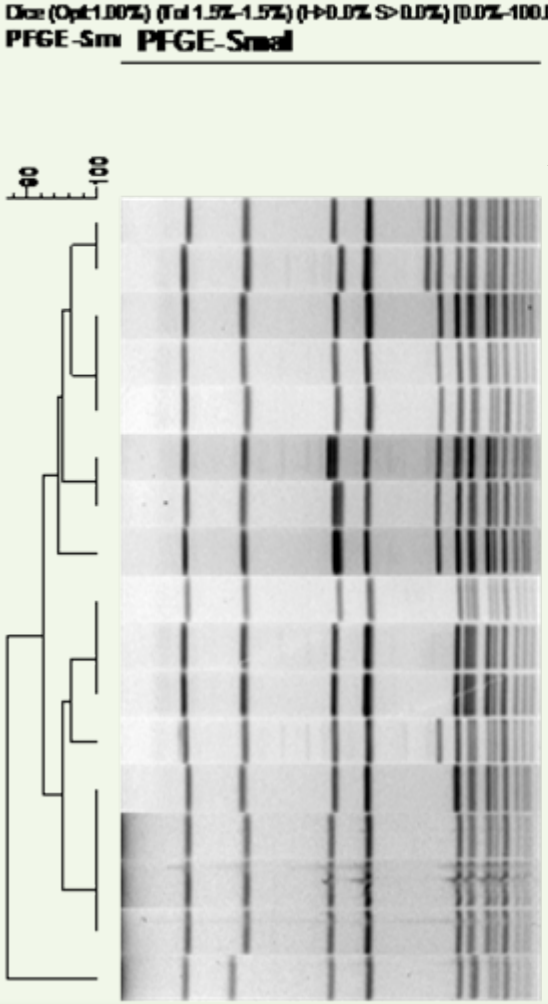


Isolate	No.	PFGE type	spa type	MLST	SCCmec
M06-4380334	31	A	t767	30	IV
M06-4380350	33	A	t019	30	IV
M06-4340027	40	A	t122	30	IV
M06-4380001	1	A	t1133	30	IV
M06-4380021	3	A	t019	30	IV
M06-4380039	5	A	t019	30	IV
M06-4380071	6	A	t019	30	IV
M06-4380074	8	A	t019	30	IV
M06-4380113	11	A	t019	30	IV
M06-4380123	13	A	t019	30	IV
M06-4380129	14	A	t019	30	IV
M06-4380132	15	A	t019	30	IV
M06-4380150	16	A	t019	30	IV
M06-4380151	17	A	t019	30	IV
M06-4380234	21	A	t019	30	IV
M06-4380249	22	A	t019	30	IV
M06-4380290	24	A	t019	30	IV
M06-4380309	28	A	t019	30	IV
M06-4340011	38	A	t019	30	IV
M06-4340042	42	A	t019	30	IV
M06-4930152	10	A	t019	30	IV
M06-4380015	2	B	t3382	30	IV
M06-4380072	7	C	t1784	88	IV
M06-4380373	35	C	t690	88	IV
M06-4340007	37	D	t852	22	IV
M06-4380329	30	E	t437	59	V
M06-4380339	32	E	t437	59	V
M06-4340003	36	E	t437	59	V
M06-4380089	9	E	t437	59	V
M06-4930284	19	E	t437	59	V
M06-4380286	23	E	t437	59	V
M06-4380291	25	E	t437	59	V
M06-4380292	26	E	t437	59	V
M06-4380368	34	E	t441	59	V
M06-4380308	27	E	t437	338	V
M06-4380034	4	E	t437	59	V
M06-4380234	29	E	t437	338	V
M06-4380116	12	E	t437	59	IV
M06-4380181	18	E	t3385	59	IV
M06-4930380	20	E	t3385	59	IV
M06-4340026	41	E	t437	59	IV
M06-4340012	39	E	t437	59	IV

PFGE types (CA-MRSA)

PFGE designation	MLST	spa type	% in HK
USA400	1	t127	0.24
USA300	8	t008	7.8
Southwest Pacific clone (HKU100)	30	t019	41.7
HKU200	59/338	t437	26.4
European	80	t044	0.32

t1081 strains (HA-MRSA)



Year-lab. no.	PFGE	spa type	SCC mec	PVL	MLST
M06-4340034	SA1S16.0164	t1081	V	-	45
M06-4340051	SA1S16.0219	t1081	V	-	45
M06-4340031	SA1S16.0196	t1081	V	-	45
M06-4340043	SA1S16.0215	t1081	V	-	45
M06-4380369	SA1S16.0026	t1081	V	-	45
M06-4340010	SA1S16.0205	t1081	V	-	45
M06-4340036	SA1S16.0213	t1081	V	-	45
M06-4340004	SA1S16.0206	t1081	V	-	45
M06-4340041	SA1S16.0053	t1081	V	-	45
M06-4340052	SA1S16.0053	t1081	V	-	45
M06-4340053	SA1S16.0053	t1081	V	-	45
SA3-0054	SA1S16.0056	t1081	V	-	45
M06-4340035	SA1S16.0186	t1081	V	-	45
M07-4340973	SA1S16.0186	t1081	V	-	
M07-4340994	SA1S16.0186	t1081	V	-	
M07-4341028	SA1S16.0186	t1081	V	-	
M07-4340019	SA1S16.0220	t1081	V	-	45

Role of laboratory

- Liaison with microbiologist for appropriate interpretation:
  - Correlation with epidemiological and clinical findings
  - Suggestion of additional testing
  - Integration of all results

Thank you