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Hetero-Vancomycin-Intermediate Methicillin-Resistant *Staphylococcus aureus* Isolate from a Medical Center in Las Cruces, New Mexico▽

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A survey of 152 methicillin-resistant *Staphylococcus aureus* (MRSA) strains from medical centers in Las Cruces, NM, and El Paso, TX, revealed the presence of *spa* types 2 and 24 (clone USA100) and *spa* type 1 (clone USA300-0114). Las Cruces MRSA displayed relatively high vancomycin MICs, and one hetero-vancomycin-intermediate *S. aureus* strain was identified.

Staphylococcus aureus is the leading cause of nosocomial infections (2), and methicillin-resistant *S. aureus* (MRSA) is a common cause of community skin and soft-tissue infections (10). The USA100 MRSA clone represents up to 44% of MRSA isolates in the United States (8), and a unique community-associated clone, USA300-0114, is widely disseminated (6, 8, 10, 17). The use of vancomycin for the treatment of serious MRSA infections is threatened by the appearance of vancomycin-intermediate *S. aureus* (VISA) and hetero-VISA (hVISA) (1, 3, 5, 18). hVISA can present initial vancomycin MICs of ≤2 μg/ml, yet upon exposure to vancomycin, VISA subpopulations appear (MICs of ≥4 μg/ml) (15, 19). VISA and vancomycin-resistant *S. aureus* (MIC of ≥32 μg/ml) are often USA100 derivatives (4, 8, 15).

A survey of two medical centers in El Paso, TX, revealed that 71% of the MRSA isolates were related USA100 clones (12). Northwest from El Paso (~50 miles) lies Las Cruces, NM (population ~80,000), a popular U.S. retirement destination. In order to understand MRSA epidemiology in a Southern New Mexico border city with a large Hispanic (~52%) and growing retirement population, we have analyzed MRSA isolates collected from Las Cruces medical centers.

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Seventy-four MRSA strains from the Memorial Medical Center and 67 MRSA strains from the Mountain View Regional Medical Center (isolated 15 September 2003 to 20 August 2004; "MM" and "MV" strains, respectively), both located in Las Cruces, ~3.5 miles apart, as well as 11 El Paso MRSA isolates ("LP" strains [12]) were analyzed (Fig. 1; Table 1). Kirby-Bauer antibiograms and fusidic acid and inducible clindamycin resistance were determined by using disc diffusion (11, 12). Vancomycin MICs were determined by using AB BIODISK E-test strips (Remel, Lenexa, KS) and agar dilution (11), and vancomycin resistance population analyses were preformed as described previously (13). VISA mutants of hVISA strain MM66 were isolated on brain heart infusion agar (Becton-Dickinson and Co., Sparks, MD) containing 3 μg/ml vancomycin (Sigma Chemical Co., St Louis, MO) by inoculating these plates with 100 μl of brain heart infusion broth culture (18 h, 200 rpm, 37°C). Pulsed-field gel electrophoresis (PFGE) of SmaI-digested DNA (14) was carried out by using the CHEF DR III system (Bio-Rad Laboratories, Inc., Hercules, CA), and SmaI restriction fragment length polymorphism similarities were determined (12). Chromosomal DNA for *spa* typing was isolated using the Qiamp DNA Mini kit (QIAGEN, Inc., Valencia, CA), and *spaA* polymorphic X regions were amplified (9) and sequenced. *spa* polymorphic X sequences were analyzed using eGenomics, and *spa* type numbers were assigned (7) (Table 1).

Five pulsed-field-type (PFT) groups containing 7 or more strains and 48 unrelated strains were identified (Fig. 1). PFT-A represented the largest PFT group (65 isolates, including 5 LP strains) and contained 13 strains of *spa* type 2 or 24, which diverge by only a single amino acid (B↔E) (Fig. 1; Table 1). PFT-B was the second-largest group (14 isolates, including 1 LP strain) (Fig. 1; Table 1) and was represented by two *spa* type 2 strains. *spa* types 2 and 24 are also represented in PFT-E, as well as four unrelated strains (MM19, MM45, MV20, and LP20), and overall these *spa* types represent 74% of the strains typed (Table 1). *spa* types 2 and 24 are representative of the multilocus sequence/SCC*mec* type ST5-MRSA-II, which identifies USA100 (16). LP57 is also *spa* type 2 (Table 1) and was previously identified as ST5-MRSA-II (12). PFT-C (nine strains) is represented by two *spa* type 1 strains, and MM2 at the PFT-C boundary and MV8 below PFT-D were also *spa* type 1 (Fig. 1, Table). *spa* type 1 is a

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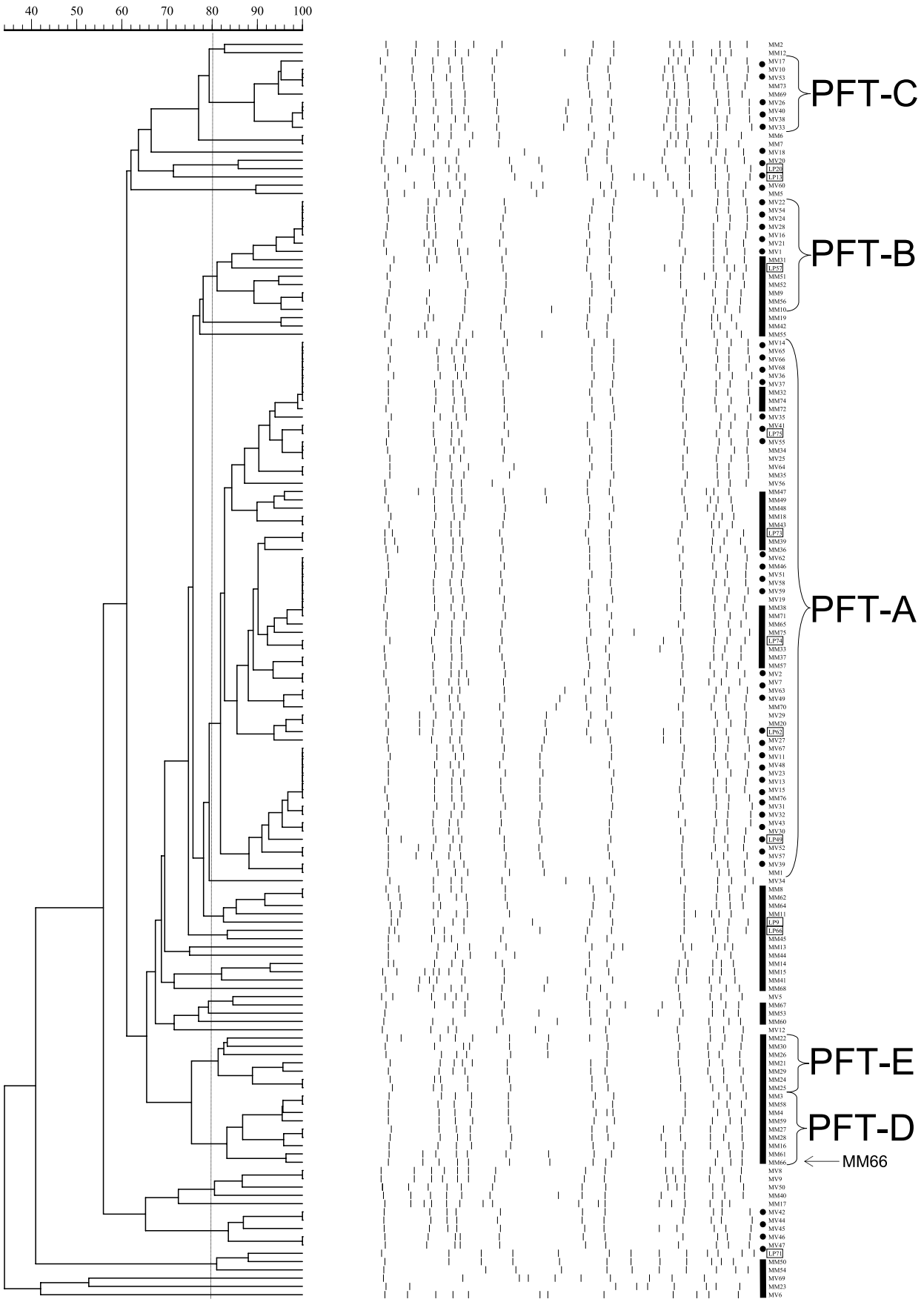
40
50
60
70
80
90
100
PFT-C
PFT-B
PFT-A
PFT-E
PFT-D
MM66

TABLE 1. Characteristics of investigated strains

Strain	Source	Antibiotype[a]	E-test MIC (μg/ml) of van[b]	Pulsotype	*spa* type; repeat
MM2	Buttock	CD*EOT	2.0		1; YHGFMBQBLO
MV53	Head	EO	2.0	C	1; YHGFMBQBLO
MV38	Body fluid	EO	1.5	C	1; YHGFMBQBLO
MV20	Urine	CDEO	2.0		24; TJMEMDMGMK
LP20	Bronchial sample	CDEGIOTm	2.0		24; TJMEMDMGMK
MM5	Thigh	O	2.0		175; UJFKPE
MV1	Wound	CDEO	1.5	B	2; TJMBMDMGMK
LP57	Wound	CD*EO	1.5	B	2; TJMBMDMGMK
MM56	Pleural sample	CD*EO	**3.0**	B	ND[c]
MM19	Sputum	CDEO	2.0		2; TJMBMDMGMK
MV66	Blood	CDEO	2.0	A	2; TJMBMDMGMK
MM32	Thigh	CEO	1.5	A	2; TJMBMDMGMK
MM74	Sputum	CD*EO	2.0	A	2; TJMBMDMGMK
MV35	Blood	CDEO	2.0	A	24; TJMEMDMGMK
LP75	Nasal	CD*EO	2.0	A	2; TJMBMDMGMK
MM49	Leg	CD*EO	**3.0**	A	ND
MM48	Bronchial sample	CD*EO	**3.0**	A	ND
MM43	Abscess	CD*EO	2.0	A	24; TJMEMDMGMK
LP73	Sputum	CDEO	2.0	A	2; TJMBMDMGMK
MM38	Leg	CDEO	**3.0**	A	ND
LP74	Sputum	CD*EOT	1.5	A	2; TJMBMDMGMK
MV49	Blood	CD*EO	2.0	A	2; TJMBMDMGMK
MM70	Back	CD*EO	2.0	A	24; TJMEMDMGMK
MV29	Urine	CDEO	2.0	A	2; TJMBMDMGMK
LP62	Wound	CD*EGOTTm	2.0	A	2; TJMBMDMGMK
MV67	Blood	CD*EO	1.5	A	24; TJMEMDMGMK
MV48	Urine	CDEIO	**3.0**	A	ND
LP66	Blood	CDEGOT	2.0		58; TJMAMGMK
MM45	Blood	CD*EO	**3.0**		24; TJMEMDMGMK
MM41	Heel	CD*EO	**3.0**		ND
MM22	Knee fluid	CD*EO	2.0	E	24; TJMEMDMGMK
MM30	Urine	CEO	1.5	E	2; TJMBMDMGMK
MM26	Foot	CD*EO	2.0	E	24; TJMEMDMGMK
MM21	Foot	CEO	**3.0**	E	ND
MM29	Sputum	CD*EO	**3.0**	E	ND
MM25	Bronchial sample	CD*EO	2.0	E	24; TJMEMDMGMK
MM4	Sputum	CEO	**3.0**	D	ND
MM59	Arm	CD*EO	**3.0**	D	ND
MM66	Sputum	CDEO	**3.0**	D	ND
MV8	Wound	DEO	2.0		1; YHGFMBQBLO
MM40	Abdomen	CD*EO	2.0		230; TMBMDMGMK
MV47	Surgical tissue	CD*EO	2.0		303; TMEMDMGMK
LP71	Unknown	CDEIMO	1.5		204; WGKAKAOMQQQQ

[a] C, ciprofloxacin; D, clindamycin; D*, inducible clindamycin resistance; E, erythromycin; G, gentamicin; I, imipenem; M, mupirocin; O, oxacillin; T, tetracycline; Tm, trimethoprim.
[b] van, vancomycin. Vancomycin E-test MICs are boldface and underlined.
[c] ND, not determined.

marker for the U.S. community-associated MRSA clone USA300-0114 (17).

PFT-D and -E are made up entirely of MM strains, and MV and MM strains throughout tended to group together in PFGE (Fig. 1). This demonstrates that while the major clones spreading within Las Cruces medical centers are similar, the clones within the two hospitals have evolved independently following introduction and/or the initial clone introduction was unique to each hospital.

All strains investigated were susceptible to synercid, linezolid, and fusidic acid, and no vancomycin-resistant *S. aureus* strains were detected. Of the 152 MRSA strains analyzed, 123 were resistant to the drugs ciprofloxacin, clindamycin, and erythromycin. Ninety percent of the Las Cruces strains demonstrated vancomycin E-test MICs of ≥2.0, and 12 exhibited a MIC of 3.0 μg/ml (Table 1). All but one of the latter group of strains (MV48) came from one medical center, and five clustered within related PFT-D and

FIG. 1. PFGE patterns of SmaI-restricted chromosomal DNA and dendrogram of percent relatedness. Bars indicate "MM" strains, and dotted lines indicate "MV" strains (if within a strain group, LP strains are included under the respective lines). "LP" strains are boxed, and MM66 is indicated with an arrow.

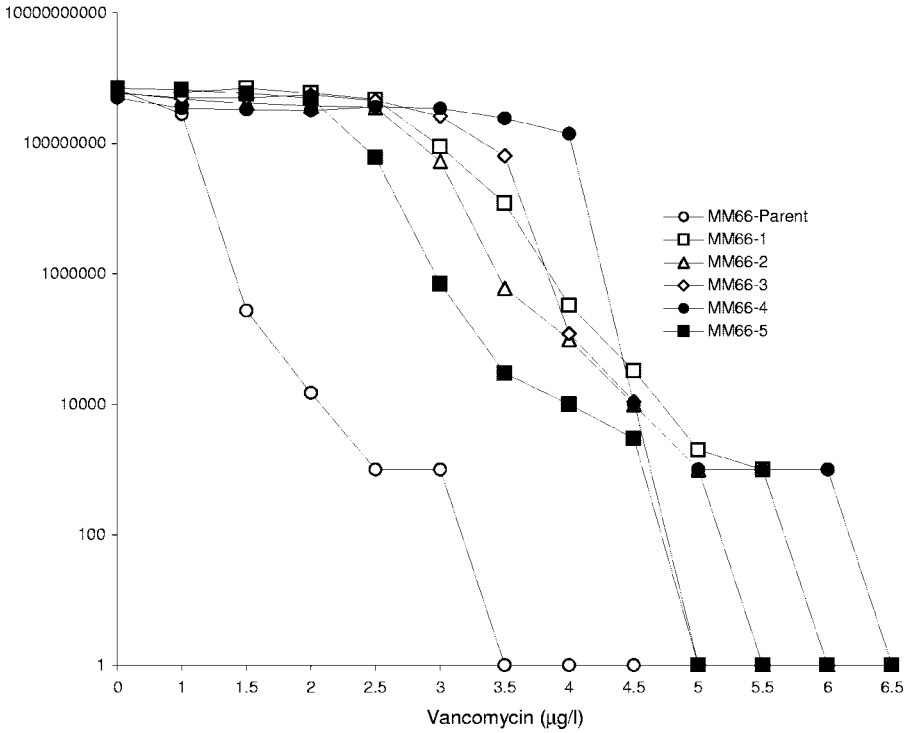

FIG. 2. Vancomycin resistance population analysis of MM66 (parent) and MM66 VISA mutants (MM66-1 through -5).

-E (Fig. 1; Table 1). The agar dilution MIC of MM66 was 4 μg/ml, and initial vancomycin resistance population analyses on all strains with E-test MICS of 3 μg/ml revealed that only MM66 produced colonies on plates containing 3 μg/ml vancomycin. hVISA strain MM66 proved 96% identical by PFT to strain MM61, yet MM61 has an additional SmaI band of 80 kb (Fig. 1) and an E-test MIC of 2.0. MM66 colonies appeared at a frequency of 1.5×10^{-6} on 3 μg/ml vancomycin, and 5 MM66 VISA colonies were picked. The agar dilution/E-test vancomycin MICs (μg/ml) for these five VISA MM66 mutants were ≥4/≥4. Colonies of hVISA MM66 did not appear above 3.0 μg/ml, while all MM66 VISA mutants produced colonies above this vancomycin concentration (4.5 to 6.0 μg/ml) (Fig. 2).

In conclusion, these findings demonstrate that USA100 clones are spreading within medical centers in two predominantly Hispanic Southwestern cities. The predominant community-associated clone USA300-0114 was also found in Las Cruces hospitals. Furthermore, we report a preponderance of MRSA with vancomycin MICs of ≥2 μg/ml in Las Cruces medical centers and have identified the first hVISA in this ethnically unique region.

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