Increasing invasive methicillin susceptible *Staphylococcus aureus* infections at Texas Children's Hospital, 2007-2010

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ABSTRACT (revised)

Almost 60% of invasive community-acquired (CA) *Staphylococcus aureus* infections were caused by MRSA (>90% USA300), at Texas Children's Hospital (TCH) from 2001-2006. During this same time we also noted a steady increase of invasive CA-MSSA infections, 25% of which were caused by USA300. While most invasive CA-MSSA USA300 isolates (25/29, 86%) were *pvl+,* only 12% (10/86) of invasive non-USA300 CA-MSSA isolates carried *pvl*.

We identified invasive CA-MSSA isolates from 2007-2010 from our *S. aureus* surveillance database. The isolates were analyzed by PFGE and by PCR for the PVL genes (*lukSF-PV; pvl*) and *arcA* (ACME cassette gene) using previously described methods. Antimicrobial susceptibilities were obtained. Statistical analyses included Fisher's exact and chi-square for trend.

179 children with invasive CA-MSSA infections were identified; 150 (84%) isolates were available for study. Overall, CA-MSSA represented 179/352 (51%) of the total invasive CA-*S. aureus* infections and the proportion of invasive isolates being MSSA increased yearly [2007- 37/95 (39%), 2008-42/86 (49%), 2009-47/90 (52%), and 2010-53/81 (65%) (p<0.001)]. Of 150 analyzed isolates, 42 were USA300 and related isolates and 108 were of other PFGE patterns. The USA300 and related isolates decreased from 11/29 (34%) isolates in 2007 to 9/45 (13%) in 2010 (p=0.03). By PCR, 92/150 (61%) isolates were *pvl+* None of the isolates carried the *arcA* gene. Of the USA300 isolates, 26 (62%) were erythromycin (E)- resistant (R) and 3 (7%) were clindamycin (C)-R; of non-USA300 isolates 23 (21%) were E-R and 14 (13%) were C-R. Mean age was 7.6 years (range 0.01-21.1), 93/150 (62%) were male. Invasive infections included osteomyelitis (98), septic arthritis (22, pneumonia/empyema (13), myositis/pyomyositis (10), bacteremia (4), lung/ retropharyngeal abscess (2), and cellulitis with bacteremia (1).

In conclusion, CA-MSSA has steadily increased as a cause of invasive infections in children at TCH and accounted for 65% of invasive *S. aureus* infections in 2010. The increasing presence of *pvl* in non-USA300 MSSA isolates suggests an advantage of these genes for the organism and may, in part, explain the rise in invasive CA-MSSA infections at TCH.

OBJECTIVES

To characterize invasive CA-MSSA, obtained at our institution between 2007-2010, by pulsed field gel electrophoresis (PFGE) and by PVL genes-PCR to evaluate the genetic relationships and trends.

INTRODUCTION

- *Staphylococcus aureus* USA300 emerged as the predominant cause of community acquired methicillin resistant *S. aureus* (CA- MRSA) infections nationwide in the early 2000's.
- USA300 MRSA, named according to its pulsed field gel electrophoresis pattern, generally carries a unique arginine catabolic mobile element (ACME), the small staphylococcal chromosome cassette *mec* (SCC*mec)* IV and the genes *lukS*-PV and *lukF*-PV, encoding for Panton-Valentine leukocidin (PVL). PVL is carried on a prophage. phiSLT is a prophage that has been shown to infect and initiate PVL production in previously PVL negative strains.
- Whether ACME or PVL significantly contributes to establishment or severity of infection remains under debate.
- At Texas Children's Hospital (TCH) we have observed the USA300 genetic background among both MRSA and MSSA isolates. A contemporary study of invasive CA- MSSA isolates showed an increase of USA300 over time from 2001-2006. Over this period, 25% of invasive CA-MSSA isolates were USA300.
- From 2001-2006 while most invasive CA-MSSA USA300 isolates (25/29, 86%) were *pvl+,* only 12% (10/86) of invasive non-USA300 CA-MSSA isolates carried *pvl.*
- We noticed a substantial increase of CA-MSSA causing invasive infections at TCH, both in direct numbers of infection and relative to the invasive infections caused by CA-MRSA. We hypothesized that the change in epidemiology would relate to an increase in USA300 CA-MSSA isolates and undertook this investigation.

METHODS

Patients were identified from a surveillance study at TCH. The study was approved by the Institutional Review Board at Baylor College of Medicine.

- TCH medical records were reviewed for demographics, duration of symptoms and hospitalization; primary diagnosis, underlying illness.
- Clinical isolates were obtained from the Clinical Microbiology Laboratory at TCH.
- Antibiotic susceptibility patterns were determined (clindamycin, erythromycin, gentamicin, oxacillin, doxycycline, tetracycline, penicillin, trimethoprim-sulfamethoxazole [TMP-SMX], vancomycin) by disk diffusion.
- Bacterial DNA was isolated using the MoBio Ultraclean Microbial DNA isolation kit. Isolates were analyzed by pulsed field gel electrophoresis (PFGE) using standard techniques and by PCR for the presence/absence of PVL genes (*lukS-PV* and *lukF-PV*) and for the Arginine Catabolic Mobile element (ACME) associated *arcA* gene using previously established techniques.
- Mann-Whitney U, Chi-square, Fisher's exact test,Chi-square for trend or log-likelihood for trend using True Epistat (Epistat Services, Richardson, TX) and STATA 10 (College Station, Texas). Analyses were 2-tailed, and a p<0.05 was considered statistically significant.

RESULTS

- 179 children with invasive CA-MSSA infections were identified from January 1, 2007 to December 31, 2010; 150 (85%) patients with isolates available were included in the molecular studies. Excluded patients/missing isolates were from: 9-2007, 5-2008, 7-2009, and 8-2010.
- CA-MSSA represented 179/352 (51%) of the total invasive CA-*S. aureus* infections and the proportion of invasive isolates being MSSA increased yearly (p<0.001). **(Table 1)**
- Of 150 analyzed isolates, 42 were grouped as USA300 and related isolates and 108 were of other PFGE patterns.

Table 1. Invasive CA-*S. aureus* isolates at Texas Children's Hospital, 2007-2010

	2007	2008	2009	2010
CA-MSSA	37 (39%)	42 (49%)	47 (52%)	53 (65%)
CA-MRSA	58 (61%)	44 (51%)	43 (48%)	28 (35%)
Total	95	86	90	81

Chi-square for trend, p<0.001

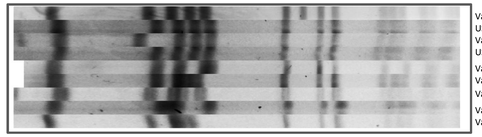
USA300 isolates

- Twenty-three isolates represented the PFGE pattern, pulsotype, that is most closely related to the MRSA USA300.0114. Ten isolates represented pulsotypes of previously recognized USA300 variants and another 9 were variants previously not observed or more divergent from the original clone. Examples of the related pulsotypes are shown in **Figure**. USA300 and variant isolates decreased as a proportion of the invasive CA-MSSA isolates from 11/29 (38%) isolates in 2007 , 14/37 (37%) in 2008, 8/39 (21%) in 2009 to 9/45 (13%) in 2010 (p=0.03).
- All isolates were PVL-PCR positive, none carried *arcA*.

Non-USA300 isolates

- Non-USA300 isolates were of diverse pulsotypes and had no resemblance to the USA300 pulsotype. No particular clone was observed to increase over time.
- PVL-PCR+ results were obtained from 50 isolates. No isolate carried *arcA*.

Figure. USA300-related pulsotypes.



Patient demographics and clinical presentation

- The most common invasive disease presentation was osteomyelitis (n=98), followed by septic arthritis (n=22), grouped as bone and joint. **(Table 2)**
- Bone and joint infections were more common among patients with non-USA300 isolates (P=0.001) and among patients with PVL genes-negative isolates (P=0.001), while pneumonia/empyema was a more common presentation for patients with a USA300 isolate (P=0.0002) and a PVL genes-positive isolate (P=0.002) .
- Patients with USA300 isolates stayed longer in the hospital compared to patients with non-USA300 isolates (P<0.00001).

Table 2. Patient characteristics

	All patients (n=150)			All patients (n=150)		
	USA300 N=42	Non-USA300 N=108	P	PVL-PCR+ 92	PVL-PCR- 58	P
Age (yrs) Median, range	6.1 (0-18)	8.9 (0-21)	0.3	7.8 (0-21)	9.4 (0-16)	0.06
Gender, n (%) Male	22 (54%)	71 (66%)	0.2	50 (54%)	43 (74%)	0.02
Race, n (%)						
Asian	1(2%)	4 (4%)	0.1	3 (3%)	2 (3%)	0.8
Black	10 (24%)	9 (8%)		14 (15%)	5 (9%)	
Caucasian	14 (33%)	45 (42%)		35 (38%)	24 (41%)	
Hispanic	12 (29%)	42 (39%)		32 (35%)	22 (38%)	
Other	5 (12%)	8 (7%)		8 (9%)	5 (9%)	
Abscess#	0	2 (2%)	>.99	2 (2%)	0	0.5
Bacteremia*	2 (5%)	3 (3%)	0.6	4 (4%)	1 (2%)	0.6
Bone & Joint	26 (62%)	94 (87%)	0.001	66 (72%)	54 (93%)	0.001
Myositis/pyomyositis	4 (10%)	6 (6%)	0.5	7 (8%)	3 (5%)	0.7
Pneumonia/empyema	10 (24%)	3 (3%)	0.0002	13 (14%)	0	0.002
No. of patients hospitalized	42	106	>.99	91	57	>.99
Hospital days, median (range)	12 (3-45)	7 (1-86)	<0.00001	9 (1-45)	8 (4-86)	0.08
No. of patients with positive blood cultures	17	35	0.4	37	15	0.08
Days with positive blood cultures, median (range)	2 (1-8)	1 (1-5)	0.004	1 (1-8)	1 (1-5)	0.9

1 lung, 1 retropharyngeal abscess, *w/cellulitis-1USA300,

Antimicrobial susceptibility

- Antibiotic susceptibility patterns are presented in **Table 3** .
- USA300-related isolates were more likely to be erythromycin resistant than non-USA300 isolates (P<0.0001).
- Clindamycin resistance rates did not differ between the two groups. However, 12/14 (86%) of the non-USA300 clindamycin resistant isolates expressed inducible resistance versus none of the USA300 isolates (p<0.0001).

Table 3. Antimicrobial resistance rates for invasive CA-MSSA isolates.

Antibiotic	USA300 N=42	Non-USA300 N=108	P
Clindamycin	3 (7%)	14 (13%)	0.4
-inducible	0	12	0.02
Erythromycin	26 (62%)	23 (21%)	<0.0001
Gentamicin	1 (2%)	0	0.28
Trimethoprim-sulfamethoxazole	0	2 (2%)	>.99
Tetracycline/doxycycline	0	2 (2%)	>.99

CONCLUSIONS

- **CA-MSSA has continued to steadily increase as a cause of invasive infections in children at TCH and accounted for 65% of CA-*S. aureus* infections in 2010. This increase was not explained by an increase of USA300.**
- **Bone and joint infections were the most common presentations, regardless of isolate type and were significantly more common among isolates with no relationship to USA300. Conversely, pneumonia was associated with USA300 isolates.**
- **46% of non-USA isolates were PVL-PCR positive by the methods used. The PVL-PCR positive strains were of various pulsotypes, marking a difference from previous results among CA-MSSA, where the PVL genes were mainly associated with a few genetic backgrounds and few strains outside of those clones carried the PVL genes. The PVL-PCR positivity observed will be further explored using expression based methods.**
- **In the USA, a study on CA-MSSA from SSTI infections reported 36% lukF-PCR+ (Kaltsas et al JCM2011). In Malaysia, 58% of MSSA isolates carried the PVL genes (Ghasemzadeh-Moghaddam et al. Int J Med Microbiol 2011).**
- **The presence of PVL genes in non-USA300 MSSA isolates may, in part, explain the rise in invasive CA-MSSA infections at TCH, and shows yet again the adaptation of *S. aureus* to new challenges by acquiring genes that provide a selective advantage. The role of PVL has not been elucidated and further research is required to better understand its role in pathogenesis.**
- **Further investigation is warranted to explain the causes of the increase of CA-MSSA invasive infections.**

This project was supported, in part, by a grant from Pfizer