Short Communication: Methicillin-Resistant *Staphylococcus aureus* Infections in Children and Young Adults Infected with HIV

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Abstract

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, in particular with Pantone-Valentine leukocidin (PVL)-positive strains, has not been well characterized in children and young adults with HIV infection. It is not known if PVL-positive strains of MRSA cause an increased morbidity in this population compared to PVL-negative strains. The purpose of this study was to retrospectively analyze the epidemiology of PVL-positive and PVL-negative MRSA infections in children and young adults with HIV from 2000 to 2007. Molecular typing was performed by polymerase chain reaction (PCR) for detection of the PVL genes. *Staphylococcus* Cassette Chromosome (SCC) *mec* and *spa* typing were performed on all PVL-positive isolates. The number of HIV patients with MRSA infection increased significantly between 2000 and 2007 ($p = 0.0015$). Twenty seven (87%) of the 31 MRSA isolates were from skin and soft tissue infections (SSTI). Clindamycin resistance was observed in 19% of the MRSA isolates. PVL-positive isolates bearing the type IV SCC *mec* element comprised 16 of 31 (52%) MRSA isolates. All the PVL-positive isolates belonged to the USA300 pulsed-field type. There was no difference in the mean CD4 count and HIV viral load between patients with PVL-positive and PVL-negative MRSA infections. PVL-positive MRSA infections were associated with more SSTI ($p = 0.043$) but not with increased morbidity or a higher risk of complications compared to PVL-negative MRSA infections in children and young adults with HIV.

Introduction

AN INCREASED INCIDENCE OF INFECTIONS with new strains of methicillin-resistant *Staphylococcus aureus* (MRSA), has been reported in children and adults worldwide over the past decade.^{1–3} Molecular typing of such isolates in the United States has shown that they are largely caused by MRSA strains that carry the Pantone-Valentine leukocidin (PVL) genes,⁴ typically the type IV staphylococcus cassette chromosome (SCC) *mec* element,⁵ and belong to USA300⁶ pulsed-field type, multilocus sequence type 8 (ST8), the most common strain causing community acquired MRSA (CA-MRSA) infections in the United States. These infections have been described in a variety of settings mostly involving otherwise healthy individuals.⁷

PVL is a pore-forming leukotoxin that has two subunits, lukS-PV and lukF-PV. Although the presence of PVL has been associated with serious infections such as suppurative pneumonia, sepsis, and necrotizing fasciitis in previously healthy children and adults,^{4,8} evidence that PVL plays a direct, causal role in the pathogenesis of these infections has been limited. While a role for PVL in dermonecrosis in rabbits has been established,⁹ evidence to show that PVL is a virulence determinant in mouse models of staphylococcal skin infections and pneumonia has been inconsistent.^{10,11}

Human immunodeficiency virus (HIV) is a known risk factor for MRSA colonization¹² and infection.¹³ Increasing rates of CA-MRSA infections have been reported among HIV-infected persons.¹⁴ These studies have been reported predominantly in adults^{15–17} and have not included children who

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have acquired HIV through perinatal infection. It is hypothesized that PVL-positive CA-MRSA strains cause more morbidity than PVL-negative strains in this population. To our knowledge, this report represents the first description of the changing epidemiology of MRSA infections in children and young adults with HIV.

Materials and Methods

The study included all children and young adults with HIV at St. Jude Children's Research Hospital (SJCRH) diagnosed with MRSA infection over an 8-year period from January 2000 to December 2007. SJCRH is a tertiary care children's research hospital with approximately 2000 outpatient visits each year by children and young adults up to 24 years of age with HIV. The institution also treats approximately 3000 patients with cancer each year. All the patients with HIV were treated exclusively at SJCRH. Patients were identified through a review of clinical microbiology culture results. The study was approved by the Institutional Review Board at SJCRH.

The number of patients with MRSA infections was determined. Data abstracted from medical record review included the following independent variables: patient age, sex, and race; history of drug abuse, incarceration, and mode of acquisition of HIV; fever at presentation; results of the blood cultures in febrile patients, duration of infection-related symptoms, and complications; CD4 cell count and viral load (VL) at diagnosis of infection; site of infection; copathogens; and antibiotic therapy and inpatient or outpatient status at time of infection. Specimen sources from which MRSA isolates originated were classified into two main groups: skin and soft tissue infections (STTI) including abscess, skin ulcers, and pustules, and other clinical sites.

Isolates were initially identified as *S. aureus* by Staphaurex plus (Remel Europe Ltd, Dartford, Kent, UK). Bacterial isolates were subsequently preserved at -80°C , with only the first isolate from each patient, per infectious episode, during the study period, included in the analysis. Recurrent infections were defined for the purpose of epidemiological analysis as positive isolates obtained from the same case 30 days or more after the last positive culture. Only the first infection was included in the analysis.

Antimicrobial susceptibility testing had previously been performed on all isolates. Screening for methicillin resistance was performed by disk diffusion using a 1- μg oxacillin disc and by growth on Mueller-Hinton agar containing 4% NaCl and oxacillin (6 $\mu\text{g}/\text{ml}$), after an incubation period of 24 h at 35°C , according to the Clinical and Laboratory Standards Institute (CLSI) methodology.¹⁸ As most of the isolates had been initially evaluated prior to the implementation of ceftioxin disk testing, all were rescreened for methicillin resistance using 30 μg ceftioxin disks (BD BB1 Sensi-Disc, Becton Dickinson, Sparks, MD). A zone <23 mm was interpreted as confirming methicillin resistance. Mupirocin resistance was confirmed using a 5- μg mupirocin disk (kindly provided by GlaxoSmithKline, Collegeville, PA). Isolates that had a zone diameter of <13 mm were considered to have low level resistance to mupirocin. Other antibiotics tested included penicillin, vancomycin, clindamycin, trimethoprim-sulfamethoxazole, erythromycin, gentamicin, and ciprofloxacin. The latter testing was performed by Vitek analysis (Vitek 32 system, BioMerieux Inc., Durham, NC). Results

were determined after 24 h of incubation at 35°C according to CLSI breakpoints. For all isolates that were erythromycin resistant and clindamycin susceptible, detection of the inducible macrolide-lincosamide-streptogramin B resistance phenotype was performed by disk diffusion with clindamycin and erythromycin disks set 15–20 mm apart (D test).¹⁸ Results of clindamycin resistance included the inducible resistance phenotype.

Frozen crude lysates of pelleted, liquid cultures were assayed by polymerase chain reaction (PCR)-based genotyping. PCR amplifications were performed using a multiplex PCR protocol previously described by Perez-Roth *et al.*¹⁹ A simultaneous detection of the *nuc* gene (encoding the thermostable nuclease of *S. aureus*) and the *mecA* gene (encoding the gene for methicillin resistance) was performed. The presence of the *lukF* and *lukS* genes of PVL was determined by PCR as previously described.²⁰ SCC *mec* typing and *spa* typing was performed on isolates that were PVL positive. The SCC-*mec* types were determined by PCR typing of the *mec* and the *ccr* gene complex as described previously by Okuma *et al.*²¹ DNA sequence-based typing of the *S. aureus* protein A (*spa*) gene was performed as previously described using template DNA extracted from overnight cultures.^{22,23} The USA pulsed-field types were inferred from the *spa* types by using the Based Upon Repeat Pattern (BURP) algorithm to cluster the *spa* types into related families or *spa* Clonal Clusters (*spaCC*). The Ridom StaphType software tool and our pulsed-field gel electrophoresis (PFGE) determinations were used to correlate the PFGE type to the *spaCC*.²⁴

Descriptive statistics, including frequencies and percentages, were obtained for the demographic and treatment variables of the patients with HIV and MRSA infection. The trend of the proportion of MRSA infection between 2000 and 2007 was evaluated by logistic regression where the odds of having an MRSA infection ($Y = 1$) over MSSA ($Y = 0$) was modeled as a function of time (time taken as a continuous variable). The trend in the proportion of PVL-positive MRSA infection between 2000 and 2007 was evaluated by logistic regression where the odds of having a PVL-positive MRSA infection ($Y = 1$) over a PVL-negative MRSA infection ($Y = 0$) was modeled as a function of time.

The association between PVL-positive MRSA infection and other independent variables was assessed by a univariate logistic regression model. The variables included age, presence of fever, viral load, CD4 counts, resistance to clindamycin or ciprofloxacin, and presence of skin and soft tissue infection. The variables that were significant at the 0.1 alpha level would enter the multiple logistic regression model. An exact logistic regression model was applied if the cell counts were less than 5. The appropriateness of the models was evaluated by the Hosmer and Lemeshow goodness-of-fit test.²⁵ To give a clearer comparison for these independent variables between PVL-positive and PVL-negative patient groups, a two-sample Wilcoxon rank sum test was performed for continuous variables and the Fisher's exact test for categorical variables. All analyses were performed in statistical software package SAS 9.1.3 (SAS Institute Inc., Cary, NC).

Results

A total of 31 MRSA isolates from clinically distinct infectious episodes were collected from 31 children and young

FIG. 1. Increasing proportion of patients with HIV and MRSA infection between 2000 and 2007 ($p = 0.0015$; p value based on the fit to logistic regression model).

adults with HIV at SJCRH over the 8-year period between January 2000 and December 2007. The number of patients with MRSA infection compared to the total number of patients with *S. aureus* infection increased from 2 out of 12 (17%) in 2000 to 4 out of 4 (100%) in 2007 (Fig. 1). The logistic fit gave a p -value = 0.0015, odds ratio 1.54 with 95% confidence interval (CI) 1.18–2.01, suggesting that the odds of having an MRSA infection over MSSA increased by 54% with each increasing year. The goodness-of-fit test gives a p -value of 0.42, which is not significant at the 5% level of significance and suggests an acceptable fit to the data. There was no significant change in the number of HIV inpatients or outpatients or in the clinical or laboratory practice at SJCRH during this 8-year period.

The demographics of this population are outlined in Table 1. Eleven of the 31 patients (35%) were children from 0 to 12 years of age, 3 (10%) were adolescents from 13 to 18 years of age, and 17 (55%) were 18 to 24 years of age. The mean duration of HIV infection was 8 years and 3 months (range: 27 months to 18 years, 6 months) and the median was 7 years and 10 months.

Of the 31 patients, 29 (94%) were outpatients and only two required hospitalization for drainage of abscesses. Of the 31 isolates collected, 27 (87%) were from skin and soft tissue infections (SSTI). Four patients had MRSA conjunctivitis. SSTI included abscesses and pustules mostly in the gluteal area and extremities. Five patients had superficial abscesses that drained spontaneously and four (13%) had deep abscesses which required incision and drainage. Only five patients (16%) had fever. None of the febrile patients was bacteremic and no patient had complications of osteomyelitis or pneumonia (Table 1).

S. aureus SSTI recurred in 3 of the 31 children (10%). Patients were followed up for recurrent infections for a mean duration of 2 years 9 months (range: 3 months to 7 years 5 months). All recurrences took place within 1 year after complete resolution of the initial infection and occurred at a site different from that of the initial infection. No patient had more than one recurrent infection. No patients suffered a relapse of infection within 30 days after discontinuation of antibiotic therapy. The viral load and CD4 counts of the 31 patients are outlined in Table 2.

HIV was perinatally acquired in 10 (32%) patients, by heterosexual exposure in 12 (39%) patients, homosexual exposure in 7 (23%) patients, bisexual exposure in 1 (3%) patient, and through transfusion in 1 (3%) patient. Of the 31 patients in the study, 8 patients (26%) had a history of incarceration or drug abuse prior to the infection. Six of the 31 patients (19%) had a history of both incarceration and drug abuse. None of the 31 patients was hospitalized or received β -lactam

TABLE 1. EPIDEMIOLOGY OF MRSA INFECTION IN 31 CHILDREN AND YOUNG ADULTS WITH HIV^a

Age, years median (range)	19 (9 months–24)
Males	17 (55)
AA race	31 (100)
Outpatients	29 (94)
Fever ($T > 38.3^{\circ}\text{C}$)	5 (16)
Complications	0 (0)

^aData are no. (%) of patients, unless otherwise indicated. MRSA, methicillin-resistant *Staphylococcus aureus*; AA, African-American.

TABLE 2. VIRAL LOAD AND CD4 COUNTS IN 31 CHILDREN AND YOUNG ADULTS WITH HIV INFECTION^a

CD4 cell count, median cells/ml (range)	560 (0–1580)
CD4 cell count <200 cells/ml	2 (6)
CD4 cell count >200 cells/ml	29 (94)
Viral load, median copies/ml (range)	784 (0–2.8 million)
Viral load <400 copies/ml	14 (45)
Viral load 400–10,000 copies/ml	6 (19)
Viral load >10,000 copies /ml	11 (35)

^aData are no. (%) of patients, unless otherwise indicated.

antibiotics in the 6 months prior to the infection and none had recurrences while incarcerated.

Resistance to ciprofloxacin, clindamycin, and erythromycin and low level resistance to mupirocin was seen in 3%, 19%, 74%, and 3% of the isolates, respectively. All isolates were susceptible to gentamicin, trimethoprim-sulfamethoxazole (TMP-SMZ), and vancomycin. Only 2 (6%) of the 31 patients were on prophylaxis with TMP-SMZ. MRSA infections were treated with either ciprofloxacin, clindamycin, or TMP-SMZ, depending on results of drug susceptibility testing.

There was no significant increase in the proportion of patients with PVL-positive MRSA infections between 2000 and 2007 ($p = 0.720$; odds ratio 0.938; CI: 0.662–1.329; p -value based on logistic regression; Fig. 2). The goodness-of-fit test gave a p -value of 0.475, suggesting an acceptable fit to the data. There were 16 PVL-positive MRSA isolates in 16 patients out of a total of 31 MRSA isolates (52%). Of the 16 PVL-positive isolates, all belonged to *spa* type 8 and were of the USA300 pulsed-field type.

Univariate analysis showed that age, presence or absence of fever, viral load, CD4 counts, and resistance to clindamycin or ciprofloxacin were not significantly different between the PVL-positive and PVL-negative patient groups. However, patients with PVL-positive MRSA infections had significantly more SSTI as compared to patients with PVL-negative MRSA infections ($p = 0.043$; exact logistic regression model and the Fisher's exact test; Table 3).

Discussion

The results show a significant increase in the number of patients with MRSA infections from 17% of all staphylococcal isolates in 2000 to 100% of isolates in 2007. Eighty-seven percent of MRSA infections were seen in the skin and soft tissue. Of the 31 patients studied, 14 (45%) were children and adolescents, while 17 (55%) were young adults. These infections caused little morbidity, were associated with fever in only 16% of patients, and did not result in any complications. Only 6% of patients had CD4 counts of less than 200 cells/ml. Forty-five percent of patients had viral loads of <400 copies/ml, while 35% had viral loads of >10,000 copies/ml. A high proportion of MRSA isolates (19%) were resistant to clindamycin. One-half of the MRSA isolates carried the PVL genes. HIV patients with PVL-positive MRSA infection were more likely to have SSTI compared to patients with PVL-negative MRSA infections.

A recent prospective study of SSTI in adults in an urban HIV clinic found a similarly high incidence of MRSA causing SSTI in 41 (93%) out of 44 patients.¹⁴ In that study, 21 (84%) of 25 isolates tested positive for SCC *mec* type IV and all tested positive for the PVL genes. The primary SSTI resolved

FIG. 2. Proportion of patients with PVL-positive MRSA infection between 2000 and 2007 ($p = 0.720$; p value based on the fit to logistic regression model).

TABLE 3. COMPARISON OF THE CLINICAL AND LABORATORY VARIABLES BETWEEN HIV PATIENTS WITH PVL-POSITIVE AND PVL-NEGATIVE MRSA INFECTIONS^a

Characteristic	PVL ⁺ (n = 16)	PVL ⁻ (n = 15)	p
Mean age (years)	14 years 4 months	15 years 9 months	0.513
Fever (T > 38.3°C)	4 (25)	1 (7)	0.333
SSTI	16 (100)	11 (73)	0.043
Viral load, median (range)	2175.5 (0–2.8 million)	784 (0–750,000)	0.704
CD4 cell count, mean (cells/ml)	721	553	0.363
Resistance to clindamycin	3 (19)	3 (20)	1.000
Resistance to ciprofloxacin	0 (0)	1 (7)	0.484

^aData are no. (%) of patients, unless otherwise indicated. PVL, Pantone-Valentine Leukocidin; SSTI, skin and soft tissue infections; *p* values derived by the Fisher's exact test for the categorical variables and exact two-sample Wilcoxon rank sum test for the age, CD4 count, and viral load.

in 37 of 40 patients (92.5%). Clindamycin resistance was seen in 10% of the isolates. As in the present series, most of the patients did not have evidence of severe immunosuppression; 49% of patients had an undetectable viral load and 76% had a CD4 cell count that exceeded 200 cells/ml. Nevertheless, recurrence of infection at a different site was seen in 11 patients (27%), as compared to 10% in the present series. Four patients (10%) in the previous study required hospitalization and three patients had secondary bacteremia, while none of our patients had any complications. Twenty of the 41 patients (49%) underwent incision and drainage, as compared to 13% in the present series. Most patients in the earlier series had risk factors for MRSA colonization including hospitalization in the 6 months prior to infection (25%), incarceration (46%), and intravenous drug abuse (32%).

CA-MRSA infections as defined by genetic typing for SCC *mec* and PVL genes were associated with high-risk sex and drug-using behaviors but not with immune status in another retrospective case-control study of HIV-positive men who had sex with men (MSM).²⁶

The present retrospective study in children and young adults is concordant with the dramatic increase in incidence of MRSA infections with the above published adult studies. However, the patients in this series experienced a lower recurrence rate of infection and a lower incidence of complications. This may be related to a lower prevalence of risk factors for MRSA colonization and infection. The increasing prevalence of MRSA infection in correctional facilities is well recognized. These patients are more likely to have received a β -lactam antibiotic in the 12 months prior to the index culture and have a higher risk of recurrence.²⁷ The reason for a higher recurrence and complication rate in the adult population is unclear but may include prolonged antibiotic therapy, which could select for resistance and continued colonization, increased risk of reinfection in correctional facilities, and high-risk sex and drug-using behaviors.

There were only seven men (23%) who acquired HIV by MSM in the present series and none of them had a history of prior hospitalization. A history of incarceration and drug abuse was noted in only 19% of patients and none had prior use of β -lactam antibiotics.

Resistance to clindamycin was seen in 19% of our population, as compared to 100% in a group of HIV adults men who had sex with men.¹⁷ Increasing resistance to clindamycin in

this high-risk population may contribute to the emergence of recurrent or persistent CA-MRSA infections.

The epidemiological association between PVL-positive MRSA infection and SSTI observed in our study is well established.⁴ Whether immune dysfunction as seen in this population affects the production of soluble PVL toxin has not been studied. However, the association between the CD4 count and the viral load with PVL-positive MRSA infection has not been consistent among investigators and was not seen in the present study.

A limitation of the study is its retrospective nature, which precludes analysis of other variables such as systemic markers of inflammation and a comparison of the clinical indicators of infection between PVL-positive and PVL-negative MRSA strains.

In conclusion, PVL-positive MRSA infections were not associated with higher morbidity or complications compared to PVL-negative MRSA infections in children and young adults with HIV. More studies with inclusion of a greater number of children perinatally infected with HIV are needed to confirm these observations.

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Disclosure Statement

No competing financial interests exist.

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