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Prevalence and Antimicrobial Agent Susceptibility of Methicillin-resistant Staphylococcus aureus in Healthy Pediatric Outpatients in Las Vegas

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Cover Page Footnote
This study was supported in part by a Planning Initiative Award from the University of Nevada, Las Vegas, NV. We thank the staff, residents, doctors, and study participants of Kids Health Pediatric Clinic and Lied Clinic of the University Medical Center, Las Vegas, NV, and Mrs. Salome K. Kapella, Program/Research Coordinator of the University of Nevada School of Medicine, Las Vegas, NV, for their assistance in the data collection process; Ms. Michelle Baker and Dr. Elliot L. Rank of BD Diagnostics, Sparks, MD, for the donation of swabs, enrichment broth and CHROMagar™ media; and Ms. Vanessa L. Stevens of the Harry Reid Center for Environmental Studies, University of Nevada, Las Vegas, NV, for her technical assistance.

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Prevalence and Antimicrobial Agent Susceptibility of Methicillin-resistant *Staphylococcus aureus* in Healthy Pediatric Outpatients in Las Vegas

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Abstract

Colonization and infection by community-associated resistant strains of *Staphylococcus aureus* are being reported in epidemic proportions. The purpose of this study was to determine the local prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in children and to characterize the MRSA isolates in the laboratory with regard to antimicrobial agent susceptibility patterns, and the presence of the *mecA* and the Panton-Valentine leukocidin (PVL) genes. Nasal swabs were collected at two pediatric clinics from a total of 505 children during health maintenance visits. A brief questionnaire was administered to collect demographic data and pertinent medical, family, and social history. Samples were cultured onto 2 selective media for *S. aureus* and MRSA. Potential MRSA isolates were further evaluated by real-time polymerase chain reaction (PCR), and for susceptibility to eight antibiotics by disk diffusion. Culture results showed that MRSA was present in 15 of the 505 specimens (3.0%). Six different antimicrobial susceptibility profiles were observed among the MRSA isolates. PCR amplification results showed that all 15 MRSA isolates were positive for the presence of the *mecA* gene, and 10 MRSA isolates contained the PVL gene. Understanding local prevalence rates and the role of colonization in infection are needed to develop effective interventions to reduce MRSA infections

Key words: community-associated MRSA, methicillin-resistant *Staphylococcus aureus*, nasal carriage, pediatric

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Colonization and infection by community-associated resistant strains of *Staphylococcus aureus* are being reported in epidemic proportions in many areas of the United States and around the world (Creech II, Kernodle, Alsentzer, Wilson, & Edwards, 2005). Although more frequently associated with skin and soft tissue infections, community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has been implicated in invasive infections in children, with wide geographical diversity in rates of colonization and infection (Hussain, Boyle-Vavra, & Daum, 2001; Kaplan et al., 2005; Kuehnert et al., 2005).

The increasing rate of MRSA infections has led many clinicians to utilize newer staphylococcal antimicrobial agents such as linezolid, as first line empiric therapy. While some studies report equal efficacy or superiority of linezolid to vancomycin in treatment of MRSA (Sharpe, Shively, & Polk Jr., 2005; Shorr, Kunkel, & Kollef, 2005), there are reports of increasing clindamycin and rifampin resistance among methicillin-resistant *Staphylococcus aureus* and of unstable vancomycin heteroresistance among clinical isolates of MRSA (Plipat, Livni, Bertram, & Thompson, 2005; Tosun et al., 2005). The two most important considerations in choosing empiric antibiotic therapy are the

knowledge of the most likely pathogen and the most likely active agent. With the increasing prevalence of MRSA, the large geographical diversity in colonization and infection rates, and the presence of increasing resistance to available therapies, clinicians should be aware of their local resistance rates.

One method of gaining this knowledge is to maintain inpatient and outpatient surveillance programs, and to identify local colonization rates and their relationship to clinical infections as well as antimicrobial resistance patterns. The anterior nares are the primary reservoir of *Staphylococcus aureus* in adults and children (Casewell, 1998; Kluytmans, van Belkum, & Verbrugh, 1997) with approximately one-third of the general population colonized at any given time (Kluytmans, van Belkum, & Verbrugh, 1997). Nasal carriage is a significant risk factor for staphylococcal infection (Davis, Stewart, Crouch, Florez, & Hospenthal, 2004; Kluytmans, van Belkum, & Verbrugh, 1997), with >80% of infecting isolates originating from the nose (vonEiff, Becker, Machka, Stammer, & Peters, 2001; Wertheim et al., 2004). In addition, eradication of nasal carriage often eliminates the organism from other body sites (Parras et al., 1995; Reagan et al., 1991).

The purpose of this study was to determine the local prevalence of MRSA nasal colonization in children. Isolates were also characterized with regard to: 1) the presence of the *mecA* gene, encoding the altered penicillin binding protein responsible for [beta]-lactam resistance; 2) antimicrobial susceptibility patterns; and 3) the presence of a specific virulence factor gene, Panton-Valentine leukocidin (PVL) (Baggett et al., 2004; Deurenberg et al., 2004; Hsu et al., 2004). This information will help in the development of recommendations for selecting antimicrobial therapy, and is the first step in maintaining a comprehensive pediatric database of the antimicrobial susceptibility of infection-causing microorganisms for Las Vegas, Nevada hospitals.

Materials and Methods

Study Design

The purpose of this study was to determine the prevalence of MRSA in healthy children aged 2 weeks to 21 years. Age ranges in this study are defined as infants (birth–2 years), adolescents (12–17 years), and young adults (18–21 years). Nasal swabs were collected from 505 children during health maintenance visits at two sites, Kids Health Pediatric Clinic and Lied Clinic of the University Medical Center in Las Vegas, Nevada. A brief questionnaire was administered by the pediatric staff to each child or responsible adult to collect demographic data and pertinent medical, family and social history. Specimens were cultured onto two selective media for the isolation of *S. aureus* and MRSA. Swabs were then enriched and subcultured onto selective media after 24 hours of incubation. Real-time polymerase chain reaction (PCR) was used to analyze MRSA isolates for the presence of *mecA* and PVL genes. The Kirby-Bauer disk diffusion method was used to further characterize isolates based on their susceptibility to eight antimicrobial agents. Data were analyzed statistically to determine MRSA prevalence rates and geospatial representation of MRSA-positivity in Clark County.

Test Organisms and Culture Media

Four bacterial reference strains were used in this study, including *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923 (a methicillin-sensitive strain), *S. aureus* ATCC 43300 (a MRSA strain), and *S. epidermidis* ATCC 12228 (American Type Culture Collection, Manassas, VA). All media were obtained from BD Diagnostics, Sparks, MD, and included BBL™ CHROMagar™ Staph aureus, BBL™ CHROMagar™ MRSA, Mueller Hinton II agar, Difco™ tryptic soy agar, Trypticase™ soy agar with 5% sheep blood, and Trypticase™ soy broth with 6.5% sodium chloride (enrichment broth). All cultures were incubated at 35°C for 24–48 hours.

Specimen Collection and Processing

Collection techniques and laboratory methods were modeled after the previous work of Nakamura (Nakamura et al., 2002). Specimens were collected by pediatric staff with a dry, sterile swab (BBL CultureSwab; BD Diagnostics) which was inserted into each nostril of each subject, rotated for 5 seconds, and placed into a tube of liquid Stuart transport medium (BD Diagnostics). Specimens were refrigerated overnight, transported to the laboratory the following morning, and processed immediately upon arrival. Swabs were streaked for isolation onto CHROMagar™ Staph aureus and CHROMagar™ MRSA, placed in enrichment broth, then vortexed for 10 seconds. All agar plate media were incubated at 35°C for 24 hours. The enrichment broth cultures were shaken at 130 rpm for 24 hours in a 35°C environmental shaker incubator. The results were recorded and CHROMagar™ MRSA plates without typical MRSA colonies were incubated for an additional 24 hours. The enrichment broth was subcultured onto CHROMagar™ Staph aureus and CHROMagar™ MRSA and streaked for isolation; plates were incubated at 35°C for 24 hours. After recording the results, the negative CHROMagar™ MRSA plates were incubated for another 24 hours. Media performance characteristics were obtained from the manufacturer's package inserts. Mauve to orange/mauve colonies produced on CHROMagar™ Staph aureus medium were identified as *S. aureus* isolates. Smooth, moderately sized mauve colonies which appeared on CHROMagar™ MRSA medium

at 24 hours were interpreted as MRSA isolates. Mauve colonies appearing at 48 hours and those with atypical growth rates or colony morphology were confirmed with further testing. These isolates were subcultured to Trypticase™ soy agar with 5% sheep blood, incubated for 24 hours, and then tested for coagulase activity (ASI Staphslide Latex Test; Arlington Scientific, Inc., Springville, UT) according to the manufacturer's instructions. All presumptive MRSA isolates were Gram stained and then stored at –70°C for use in additional testing.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion (direct method) on Mueller Hinton medium according to Clinical and Laboratory Standards Institute (Wayne, PA) standards and included the following antimicrobial agents: clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), mupirocin (5 µg), rifampin (5 µg), trimethoprim-sulfamethoxazole (1.25 µg/23.75 µg), and vancomycin (30 µg). All antimicrobial agents were obtained from Becton Dickinson, except mupirocin, which was obtained from Mast Diagnostics, Merseyside, UK. Organisms included in testing for quality control purposes were *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Presumptive MRSA isolates were subcultured onto tryptic soy agar and incubated for 24 hours. Each bacterial suspension was prepared according to the antimicrobial agents manufacturers' instructions by inoculating 5 ml of Bacto™ tryptic soy broth (BD Diagnostics) with a few isolated colonies and diluting the suspension as needed to obtain turbidity equivalent to a 0.5 McFarland standard (Wanger, 2007). Within 15 minutes of preparation, a sterile cotton swab was dipped into the suspension; excess liquid was expressed from the swab. The agar surface was inoculated three times, rotating the plate 60° each time, then sweeping the swab around the outer rim of the agar. The plates were allowed to dry for 3–5 minutes before applying the disks. Four disks were placed manually onto each plate with sterile forceps and pressed firmly onto the surface. To detect inducible clindamycin resistance, double disk diffusion testing (D test) was performed by placing the clindamycin and erythromycin disks 15 mm apart (center to center); all other disks were placed 30 mm apart (Fiebelkorn, Crawford, McElmeel, & Jorgensen, 2003; Frank et al., 2002). Plates were incubated aerobically at 35°C for 16–18 hours. Zone diameters were measured to the nearest millimeter and results were recorded as susceptible, intermediate, or resistant based on interpretive criteria provided by the antimicrobial agents manufacturers.

DNA Extraction and Purification

Several colonies from each MRSA isolate were suspended in 0.5 ml 0.01 M potassium phosphate buffer with 0.05% Tween 20 (Sigma-Aldrich Co., St. Louis, MO) (pH 7.0). DNA extraction and purification from all test and control cultures were performed using previously published protocols (Buttner et al., 2004). Briefly, each suspension was pretreated with sodium dodecyl sulfate and proteinase K, incubated at 50°C, then boiled and chilled on ice. Bovine serum albumin was added, followed by incubation at 37°C in a rotary shaker. The DNA from all isolates was purified by using the Pellet Paint protocol (Novagen, Madison, WI). Pellets were air dried overnight, then resuspended in 50 µl of Tris-EDTA buffer (pH 8.0) and stored at –70°C.

Polymerase Chain Reaction

A real-time PCR assay utilizing the TaqMan® (Applied Biosystems, Foster City, CA) technology was used to detect the *mecA* gene (Francois et al., 2003). The 7900 HT Fast Real-Time PCR System (Applied Biosystems) was used for PCR analysis. All primers were obtained from Operon Technologies (Huntsville, AL) and the probes were obtained from Applied Biosystems. Presumptive isolates were analyzed using the oligonucleotide primers and probe specified previously (Francois et al., 2003). The *mecA* primer sequences (5′ to 3′) were as follows: cattgatcgcaacgttcaattt (forward) and tggtctttctgcattcctgga (reverse). The TaqMan® probe sequence was 6-FAM-tggaagttagattgggatcatagcgtcat-TAMRA. The reaction conditions included: 5 µl genomic DNA, 1X TaqMan® Universal PCR Master Mix (Applied Biosystems), 100 nM of each primer, and 75 nM of probe. The reaction volume was adjusted to 25 µl with nuclease-free water (Promega Corp., Madison, WI). The TaqMan® amplification conditions in Standard mode were 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 sec at 95°C followed by 1 min at 60°C. Isolates were further analyzed for the PVL gene by real-time PCR with the use of oligonucleotide primers and probe sequences as described previously (Deurenberg et al., 2004). The PVL primer sequences (5′ to 3′) were: gctggacaaaacttcttggaatat (forward) and gataggacaccaataaattctggattg (reverse). The TaqMan® probe sequence was 6-FAM-aaaatgccagtgttatcca-MGBNFQ. The reaction conditions included 5 µl genomic DNA, 1X TaqMan® Universal PCR Master Mix, 0.6 µM of each primer, and 175 nM MGB probe. The reaction volume was adjusted to 25 µl with nuclease-free water. The amplification conditions were as described above. All MRSA isolates were amplified in duplicate, and negative and positive controls were

included with each PCR assay. DNA for both *mecA* and PVL genes was assayed both undiluted and diluted 10-fold. In addition, a 100-fold dilution was assayed for the PVL gene. After amplification, the data were analyzed and plotted (fluorescence vs. cycle number) using the software provided with the 7900 PCR System. The extent of amplification was reported by the software as the mean C_T value of two replicates. C_T refers to the PCR cycle number where detectable amplification product is measured, and is inversely proportional to the initial DNA template concentration.

Statistical Methods and Analyses

The number of study participants was determined based on the population of 20,000 patients between the ages of 2 and 21 years that utilize the two clinics (14,000 at the Lied Clinic of the University Medical Center, and 6,000 at the Kids Health Pediatric Clinic). An estimated MRSA colonization rate of 2.4% (a three fold increase from the average national rate of 0.8%) and a hypergeometric distribution (sampling without replacement – i.e., patients sampled only once throughout the study) were utilized. This resulted in an estimate that 475 participants were needed to provide a population MRSA colonization prevalence rate with 95% confidence and a power of 0.80 (alpha = 0.05; Fisher exact test method). Descriptive and inferential statistics were calculated for all variables using SPSS version 15.0, SAS version 9.1, and NCSS/PASS 2004.

Results

Culture Analysis

A total of 505 nasal swabs were obtained from the two pediatric clinics. Results of culture analysis showed that 15 of the 505 specimens (3.0%) were positive for the presence of MRSA. Ten positive specimens were obtained from the Kids Health Pediatric Clinic and five positive specimens were obtained from the Lied Clinic of the University Medical Center. Of the MRSA positive specimens, 5 were obtained 24 hours after direct inoculation onto CHROMagar™ MRSA, 1 was obtained 48 hours after direct inoculation, and 9 were obtained after enrichment for 24 hours. Incubation of the plated enrichment broth for 48 hours yielded no additional positive results. Although mauve colonies appeared on 13 of the enriched specimens at 48 hours, these were ruled out as potential MRSA by negative coagulase tests.

S. aureus was isolated with CHROMagar™ Staph aureus in a total of 177 of 505 specimens (35.0%). The prevalence of MRSA among *S. aureus* isolates was 8.5%. Of the positive *S. aureus* specimens, 115 were obtained by direct inoculation and 62 were detected only after enrichment. For 2 specimens in which positive results were obtained by direct inoculation (1 and 2 CFU, respectively), enrichment results were negative. All positive MRSA specimens obtained with CHROMagar™ MRSA were also positive for *S. aureus* on CHROMagar™ Staph aureus.

Questionnaire Data

A total of 498 questionnaires were obtained with the 505 specimens. All positive MRSA specimens were accompanied by completed questionnaires. The number of respondents between categories varied due to incomplete or missing information obtained for some questions. Basic summary statistics from the questionnaire responses from patients showed a median age of 4.9 and 1.9 years of age for Kids Health Pediatric Clinic (N = 230) and Lied Clinic (N = 251), respectively. Combined demographics for both clinics showed that 4.3% (21/488) of the participants had lived in a group home in the past year, 5.0% (25/498) had a household member working in a hospital or clinic, and 1.2% (6/498) had a household member working in a correctional facility. The medical history of the participants from both clinics revealed that 68 of 490 (13.9%) had a history of chronic illness, 86 of 488 (17.6%) had been hospitalized, and 24 of 408 (5.9%) had been admitted to the intensive care unit of a hospital. The antibiotic use history of the participants indicated that 219 of 478 (45.8%) had been treated with antibiotics, 136 of 409 (33.3%) in the past year, and 42 of 405 (10.4%) in the last month. Questionnaire responses from adolescents and young adults (ages 12–21), revealed that 1 in 65 (1.5%) had used intravenous illegal drugs or done skin popping, 3 in 66 (4.5%) had tattoos, and 2 in 60 (3.3%) had been in jail or juvenile detention.

Analysis of the questionnaire data from families with children colonized by MRSA showed a median age of 3.2 and 0.6 years of age for Kids Health Pediatric Clinic and Lied Clinic, respectively (Table 1). Combined demographics for both clinics showed that of 15 patients, one had lived in a group home in the past year, 3 had a household member working in a hospital or clinic, and 1 had a household member working in a correctional facility (Table 1).

Table 1. Demographics of MRSA positive patients seen at Kids Health Pediatric Clinic and Lied Clinic, Las Vegas, NV as reported on the study questionnaire by each child or responsible adult (S.D. = standard deviation).

Variable	Clinic	N	Mean	SD	Median	Minimum	Maximum
Age (yrs)	Kids Health	9	5.0	5.56	3.2	0.5	15.4
	Lied Clinic	5	4.6	7.09	0.6	0.3	16.8
Total People in Home	Kids Health	9	5.4	2.24	5.0	2.0	9.0
	Lied Clinic	5	5.8	1.92	6.0	3.0	8.0

Variable	Category	Kids Health (N = 10)		Lied Clinic (N = 5)	
		Frequency	Percent	Frequency	Percent
Gender	Female	2	20.0	3	60.0
	Male	8	80.0	2	40.0
City	North Las Vegas	3	30.0	1	20.0
	Henderson	1	10.0	0	0.0
	Las Vegas	6	60.0	4	80.0
School	Daycare	1	10.0	1	20.0
	Elementary	1	10.0	0	0.0
	Middle/High	2	20.0	1	20.0
	None	6	60.0	3	60.0

Question	Kids Health			Lied Clinic		
	N	No	Yes	N	No	Yes
Has participant lived in group home in past year?	10	**10** (100%)	**0**(0%)	5	**4** (80%)	**1** (20%)
Does any member of the participant's household work in a hospital/clinic?	10	**7** (70%)	**3** (30%)	5	**5** (100%)	**0** (0%)
Does any member of the participant's household work in a chronic care facility?	10	**10** (100%)	**0** (0%)	5	**5** (100%)	**0** (0%)
Does any member of the participant's household work in a school?	10	**7** (70%)	**3** (30%)	5	**4** (80%)	**1** (20%)
Does any member of the participant's household work in a daycare?	10	**10** (100%)	**0** (0%)	5	**5** (100%)	**0** (0%)
Does any member of the participant's household work in a correctional facility?	10	**10** (100%)	**0** (0%)	5	**4** (80%)	**1** (20%)
Does any member of the participant's household work in a home/school for disabled persons?	10	**10** (100%)	**0** (0%)	5	**5** (100%)	**0** (0%)

The medical history indicated that 5 of the 15 children had been hospitalized (Table 2). The antibiotic use history of these children indicated that 10 of 15 (66.7%) had been treated with antibiotics, 9 of 13 (69.2%) in the past year, and 4 of 12 (33.3%) in the last month (Table 3). Questionnaire responses from MRSA positive adolescents and young adults (N = 4) revealed that one had used intravenous illegal drugs or done skin popping, and had been in jail or juvenile detention (Table 4).

Table 2. Medical history of MRSA positive patients seen at Kids Health Pediatric Clinic and Lied Clinic, Las Vegas, NV as reported on the study questionnaire by each child or responsible adult (NICU = neonatal intensive care unit).

Question	Kids Health			Lied Clinic		
	N	No	Yes	N	No	Yes
Does the participant have a history of chronic illness?	10	**9** (90%)	**1** (10%)	5	**4** (80%)	**1** (20%)
Has the participant been hospitalized?	10	**7** (70%)	**3** (30%)	5	**3** (60%)	**2** (40%)
Does any member of the participant's household have a history of chronic illness?	10	**9** (90%)	**1** (10%)	5	**4** (80%)	**1** (20%)
If an infant, did s/he spend a prolonged time in a nursery or NICU?	2	**2** (100%)	**0** (0%)	1	**1** (100%)	**0** (0%)

Table 3. Antibiotic use history of MRSA positive patients seen at Kids Health Pediatric Clinic and Lied Clinic, Las Vegas, NV as reported on the study questionnaire by each child or responsible adult.

Question	Kids Health			Lied Clinic		
	N	No	Yes	N	No	Yes
Has the participant ever been treated with antibiotics?	10	**3** (30%)	**7** (70%)	5	**2** (40%)	**3** (60%)
Has the participant been treated with antibiotics in the past year?	9	**3** (33%)	**6** (67%)	4	**1** (25%)	**3** (75%)
Has the participant been treated with antibiotics in the last month?	8	**5** (62%)	**3** (38%)	4	**3** (75%)	**1** (25%)
Has any member of the participant's household used antibiotics?	0	**0** (0%)	**0** (0%)	0	**0** (0%)	**0** (0%)
Has any member of the participant's household been treated with antibiotics in the past year?	9	**3** (33%)	**6** (67%)	4	**2** (50%)	**2** (50%)
Has any member of the participant's household been treated with antibiotics in the last month?	9	**5** (56%)	**4** (44%)	5	**5** (100%)	**0** (0%)

Table 4. MRSA positive adolescent and young adult (12–21 year-old) basic summary statistics from Kids Health Pediatric Clinic and Lied Clinic, Las Vegas, NV as reported on the study questionnaire by each child or responsible adult (IV = intravenous).

Question	Kids Health			Lied Clinic		
	N	No	Yes	N	No	Yes
Do you smoke cigarettes?	3	**3** (100%)	**0** (0%)	1	**1** (100%)	**0** (0%)
Have you ever used IV illegal drugs or done skin popping?	3	**3** (100%)	**0** (0%)	1	**0** (0%)	**1** (100%)
Do you have tattoos?	3	**3** (100%)	**0** (0%)	1	**1** (100%)	**0** (0%)
Do you have pierced ears?	3	**1** (33%)	**2** (67%)	1	**1** (100%)	**0** (0%)
Do you have piercings in any other parts of your body?	2	**2** (100%)	**0** (0%)	1	**1** (100%)	**0** (0%)
Do you work?	3	**3** (100%)	**0** (0%)	1	**1** (100%)	**0** (0%)
Do you have a boyfriend/girlfriend?	3	**3** (100%)	**0** (0%)	1	**1** (100%)	**0** (0%)
Have you ever been in juvenile detention or jail?	1	**1** (100%)	**0** (0%)	1	**0** (0%)	**1** (100%)

Antimicrobial Susceptibility Testing

Six different antimicrobial agent susceptibility profiles were observed from the MRSA isolates (Table 5). Four of the isolates were susceptible to all eight antimicrobial agents tested. Conversely, one isolate was resistant to four antimicrobial agents. Resistance to erythromycin was most frequent, followed by clindamycin resistance. Inducible clindamycin resistance due to erythromycin was observed for two isolates.

Table 5. Antimicrobial agent susceptibility results obtained for MRSA positive specimens. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as QC organisms. Antimicrobial agent susceptibility [susceptible (S), intermediate (I), and resistant (R)] was determined with the Zone Diameter for *Staphylococcus* spp. Interpretive Standards (CC = Clindamycin, 2 µg; E = Erythromycin, 15 µg; GM = Gentamicin, 10 µg; LZD = Linezolid, 30 µg; RA = Rifampin, 5 µg; SXT = Trimethoprim/ Sulfamethoxazole, 1.25 µg/23.75 µg; Va = Vancomycin, 30 µg; MUP = Mupirocin, 5 µg).

Specimen	Antimicrobial Agent							
	CC	E	GM	LZD	RA	SXT	Va	MUP
0085	S	S	S	S	S	S	S	S
0086	S	S	S	S	S	S	S	S
0111	**R**	**R**	S	S	S	S	S	S
0124	**R**	**R**	**R**	S	S	**R**	S	S
0141	**R**	**R**	S	S	S	S	S	S
0143	**R**	**R**	S	S	S	S	S	S
0185	S	**R**	S	S	S	S	S	S
0203	S	**R**	S	S	S	S	S	S
0204	S	**R**	S	S	S	S	S	S
0234	**R**[a]	**R**	S	S	S	S	S	S
L084	S	**R**	S	S	S	S	S	S
L143	S	S	S	S	S	S	S	S
L157	S	**R**	S	S	S	S	S	S
L226	**I**[a]	**R**	S	S	S	S	S	S
L039	S	S	S	S	S	S	S	S

[a] D-zone effect observed between clindamycin and erythromycin

Polymerase Chain Reaction Analysis

PCR results are summarized in Table 6. All MRSA isolates were confirmed by positive amplification results for the presence of the *mecA* gene. Ten of the fifteen MRSA isolates contained the PVL gene, including all five isolates obtained from children who had been hospitalized. Dilutions of the DNA were performed for all samples, and sometimes produced a stronger amplification result (lower cycle threshold value, C_T), indicating that partial PCR inhibition occurred due to high concentrations of DNA in the specimen (data not shown).

Table 6. PCR results obtained for the amplification of MRSA isolates. Targets consisted of the PVL and *mecA* genes. Plus (+) signs represent positive amplification, and minus (–) signs represent no amplification. Two replicates were amplified for each DNA sample (ATCC = American Type Culture Collection).

Microorganism	QPCR Results	
	PVL gene	*mecA* gene
QC Organism		
Escherichia coli ATCC 25922	–	–
Staphylococcus aureus ATCC 25923	+	–
S. aureus ATCC 43300[a]	–	+
S. epidermidis ATCC 12228	–	–
Specimen Id. #		
0085	+	+
0086	+	+
0111	–	+
0124	–	+
0141	–	+
0143	–	+
0185	+	+
0203	+	+
0204	+	+
0234	+	+
L039	+	+
L084	+	+
L143	+	+
L157	+	+
L226	–	+

[a] MRSA strain

Discussion

Studies on MRSA colonization in healthy pediatric populations have indicated nasal carriage rates ranging from 0.2 to 2.5% (Cheng Immergluck et al., 2004; Hussain, Boyle-Vavra, & Daum, 2001; Kuehnert et al., 2006; Mainous III, Hueston, Everett, & Diaz, 2006; Nakamura et al., 2002; Shopsin et al., 2000). The MRSA colonization prevalence of 3.0% measured for children in this study lies outside this range and is considerably greater than the estimate of 0.6% (1–19 year age group) obtained in a nationwide survey conducted in 2001–2002 (Kuehnert et al., 2006). Because colonization typically precedes infection, it is probable that the epidemic of MRSA infections is a result of increasing colonization rates in the population nationwide. Two studies conducted in Nashville, Tennessee in 2001 and 2004 showed that the colonization rate in children had increased from 0.8% to 9.2% in three years (Creech II, Kernodle, Alsentzer, Wilson, & Edwards, 2005; Nakamura et al., 2002). Although this is the first known survey of MRSA colonization of healthy

children in Las Vegas, the results support the premise that MRSA colonization rates are increasing. While the number of MRSA specimens was relatively small, the sample size was sufficiently large to estimate the population prevalence rate with 95% confidence. However, the small number of positives precludes any further comparisons of questionnaire data other than descriptive ones. *S. aureus* was present in 35.0% of the specimens, similar to the estimate of 36.9% (1–19 year age group) obtained in the nationwide survey (Kuehnert et al., 2006), and to the prevalence of 36.4% observed in Nashville children in 2004 (Creech II, Kernodle, Alsentzer, Wilson, & Edwards, 2005).

The presence of the *mecA* gene is considered an indicator of methicillin resistance in *Staphylococcus aureus* (Francois et al., 2003), and all of the positive results obtained with the selective medium, CHROMagar™ MRSA, were confirmed by positive PCR results for the *mecA* gene. Previous research has indicated that CHROMagar™ MRSA has high specificity and sensitivity for the detection of MRSA (Flayhart et al., 2005; Perry et al., 2004). The data obtained from this survey showed that the enrichment step in the culture protocol could improve the detection of MRSA, as 9 of the 15 MRSA isolates were detected by this step. Of the 6 MRSA isolates detected by direct inoculation, 5 were isolated after 24 hours of incubation and 1 was determined after 48 hours. Because the enrichment broth step amplifies those potential pathogens in numbers too low to recover by routine culture techniques, it is a procedure that individual laboratories can choose to perform to enhance the detection of MRSA, especially in low prevalence populations. However, the disadvantages of the enrichment broth step are the increase in the time delay to the determination of a negative result, and increased costs. Therefore, this optional procedure may be considered by laboratorians depending upon their individual circumstances.

MRSA infections that are acquired by persons who have not been recently hospitalized or have had a medical procedure are termed CA-MRSA infections. On the basis of questionnaire data, 10 of the 15 isolates in this study appear to be CA-MRSA. Further molecular typing is needed to characterize the MRSA strains isolated in this study and determine whether they are consistent with healthcare-associated or CA-MRSA. The *mecA* gene is located on a mobile genetic element, SCC*mec* (staphylococcal cassette chromosome *mec*) (Katayama et al., 2003). There are several different SCC*mec* elements that have been characterized, with CA-MRSA strains typically carrying the Type IV SCC*mec* element (Huang et al., 2006; Moroney, Heller, Arbuckle, Talavera, & Widen, 2007). The PVL gene is associated with virulence and was initially thought to be a characteristic of CA-MRSA strains. However, one study showed that the PVL gene was present in only 9.3% of MRSA isolates containing the type IV SCC*mec* (Francois et al., 2004). In this study, the PVL gene was present in all 5 apparent healthcare-associated MRSA isolates. The frequency of occurrence of the PVL gene was high among MRSA isolates in this study (66.7%) compared with the nationwide survey in which 8% of MRSA isolates contained the gene (Kuehnert et al., 2005), and the 22% frequency observed in another study (Creech II, Kernodle, Alsentzer, Wilson, & Edwards, 2005). One of the limitations of this study was that the MRSA isolates were not fully characterized according to SCC*mec* strain types. However, that was not a stated objective of this initial survey.

Four of the MRSA isolates were sensitive to all 8 antimicrobial agents tested. Resistance to erythromycin was most prevalent (11 of 15), followed by clindamycin (5 of 15). One isolate demonstrated resistance to 4 antimicrobial agents. These results have clinical implications for treatment of MRSA infections. Physicians treating pediatric patients with skin and soft tissue infections should consider use of empiric antimicrobial agents with broad spectrum against MRSA until more susceptibility data are available for developing recommendations for antimicrobial therapy. Additional monitoring and research are needed to provide a more comprehensive estimate of the prevalence and types of MRSA occurring in the local pediatric population. Expanded surveillance utilizing strain typing will permit tracking of MRSA isolates in community acquired and nosocomial infections. This will provide information that can be used to minimize transmission and reduce the incidence of MRSA in children in Las Vegas.

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