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Comparison of the Impact of Colonization by Methicillin-Resistant *Staphylococcus aureus* with Different SCC*mec* Cassettes on Pediatric Cystic Fibrosis Patients

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Background

The increased prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) stains over the past 30 years have affected the Cystic Fibrosis (CF) population by increasing colonization rates from 11.9% in 2003 to 25.6% in 2013[1]. The presence of MRSA in CF lungs causes a decrease in lung function when compared to CF patients colonized with methicillin-sensitive *S. aureus* (MSSA)[2-5]. These studies indicated that MRSA is more virulent than MSSA in this patient population.

Additionally, different strains of MRSA may impact CF lung health adversely. In the United States, MRSA strains typically carry the *mec*A gene on either the SCC*mec* II or SCC*mec* IV cassette. SCC*mec* II strains emerged in the hospital setting and are typically multi-drug resistant while SCC*mec* IV strains are thought to have arisen from MSSA strains in the community. There have been limited studies examining the effect of MRSA strain type colonization on the CF population.

This study examined a cohort of CF patients colonized with MRSA strains containing either an SCC*mec* II or SCC*mec* IV cassette to determine whether MRSA strain type affects health outcomes. Also, the MRSA isolates were genetically analyzed in order to identify the presence of virulence genes and to compare the two strains to determine if one type is more virulent than the other. Antibiotic resistance patterns were compared along with prescribed courses of antibiotics to determine differences in treatment.

Methods

Study Design:

Isolates from clinical respiratory cultures identified as MRSA were collected on pediatric CF patients from April 2014 to February 2015 and analyzed using whole genome sequencing technology. Initial MRSA colonization date was determined using a standard definition. Patients were grouped based on SCC*mec* cassette type; SCC*mec* II or SCC*mec* IV. Ten patients from the SCC*mec* IV group were matched by age and gender with ten patients with SCC*mec* II isolates. A retrospective case-control study was developed using a one year pre- and post-MRSA acquisition design.

Patient Data Collected:

- ♦ number of hospitalizations
- ♦ pulmonary exacerbations
- ♦amount/type of clinic visits
- ♦ Body Mass Index percentile
- ♦ FEV_1%
- ♦ courses of oral antibiotics

MRSA Isolate Analysis

- Organism identification → Vitek MS+ system
- Antimicrobial susceptibility testing → Vitek 2 system
- Whole Genome Sequencing → Illumina HiSeq 2000
- Virulence gene identification → BowTie2
- MRSA reference genome sequences → RefSeq database

Data Analysis

All statistical analyses were performed used SAS Enterprise 9.3. The Wilcoxon rank sum two sample test was used for non-parametric data. Repeated measures were analyzed using a linear mixed model approach by group and time. Parametric data was analyzed with Poisson regression and Fisher's Exact test. Analysis was two-tailed and statistical significance was accepted at p <0.05 using a 95% confidence interval.

Results

During the study period, 50 CF patients met the study definition for MRSA colonization with 26 isolates containing the SCC*mec* II cassette and 24 isolates containing the SCC*mec* IV cassette. Only 10 patients met the study inclusion criteria from the SCC*mec* IV group and were matched by age and gender with 10 patients from the SCC*mec* II group.

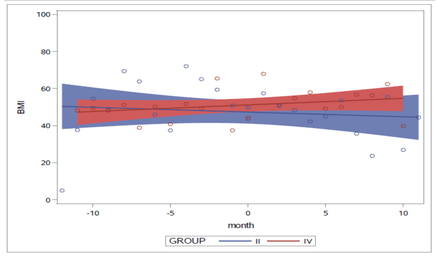
Demographics of Study Participants

Characteristic	SCCmec II N=10	SCCmec IV N=10
Average age at acquisition in years (range)	11 [6 – 20]	11 [6 – 21]
Male/Female	4/6	4/6
Pseudomonas aeruginosa	2 (20%)	1 (10%)
F508del (CF genotype)	7 (70%)	9 (90%)
Median year of MRSA colonization (range)	2011 (2007-2014)	2013 (2010-2014)

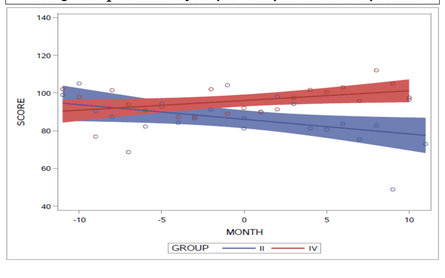
Results of Health Measurement Comparison

Health Assessment Measure	SCCmec II	SCCmec IV	p value
Number of hospitalizations 1 year prior MRSA acquisition	13	4	
Number of hospitalizations 1 year post MRSA acquisition	7	3	0.50
Number of pulmonary exacerbations 1 year prior MRSA acquisition	16	3	
Number of pulmonary exacerbations 1 year post MRSA acquisition	15	5	0.51
Average BMIp 1 year prior MRSA acquisition	54.9% ±24.6%	47.9% ±14.3%	0.02
Average BMIp 1 year post MRSA acquisition	45.4% ±27.2%	51.7% ±15.6%	
Average FEV1% 1 year prior MRSA acquisition	89.9% ±15.9%	92.9% ±11.2%	0.001
Average FEV1% 1-year post MRSA acquisition	84.8% ±18.2%	97.9% ±14.8%	

Average BMIp scores 1 year pre- and post-MRSA Acquisition

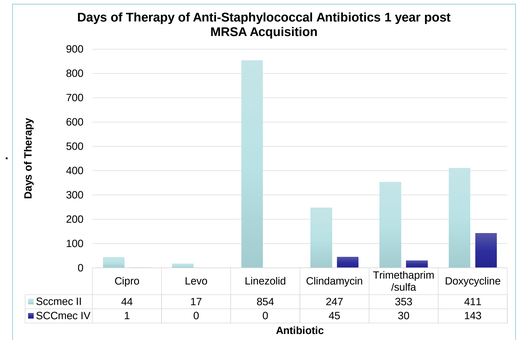


Average FEV_1% scores 1 year pre- and post-MRSA Acquisition

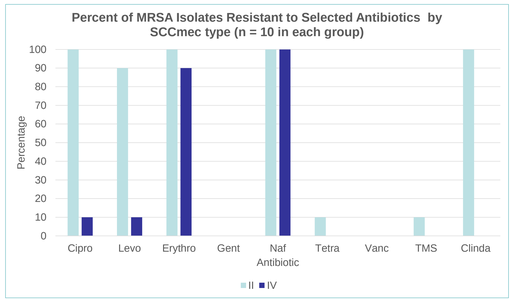


Results

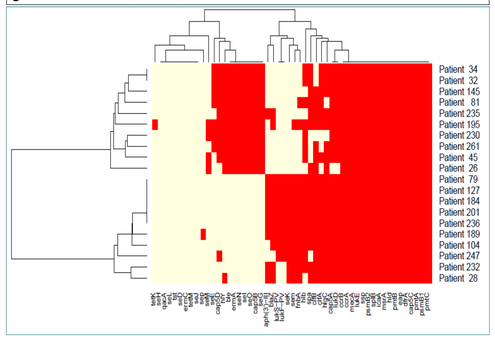
Days of Therapy of Anti-Staphylococcal Antibiotics 1 year post MRSA Acquisition



Percent of MRSA Isolates Resistant to Selected Antibiotics by SCCmec type (n = 10 in each group)



Phylogenetic Heat Map of Virulence genes: red indicates presence of the gene listed on horizontal axis



Comparison of the Presence of Important Virulence Genes

Gene	SCCmec II	SCCmec IV
PVL	0	7
Leukocidin D and E	10	10
A-hemolysin	10	1
Enterotoxins (seB, seC, seE, seG, sel, seM, seN, seO)	10	0
seK	0	10
cap5B, cap5D	10	1

Conclusions

1. SCC*mec* II MRSA strains do affect health outcomes in CF patients in the first year post-acquisition when compared to CF patients with SCC*mec* IV MRSA.
2. SCC*mec* II MRSA strains have the potential to be more virulent then SCC*mec* IV MRSA due to the presence of extra virulence genes.
3. The SCC*mec* II MRSA isolates in this study were more drug resistant then the SCC*mec* IV MRSA isolates and these patients received more antibiotics in the first year post-MRSA acquisition.
4. It is relevant to identify MRSA SCC*mec* type in CF patients in order to understand the potential clinical impact.

Future Studies

1. Develop a predictive model based on antimicrobial sensitivities to identify strains as SCC*mec* II MRSA isolates in the CF population in the absence of molecular testing.
2. Research the possible virulence of the *Staphylococcal* Enterotoxins present in the SCC*mec* II MRSA isolates to determine any impact on CF lungs.

Study Limitations

1. Due to the small sample size, the statistical power may be limited.
2. The colonization date was estimated (1st positive culture) and does not represent the true moment of MRSA acquisition.
3. The presence of other CF pathogens was not taken into consideration – most CF patients of this age group are not yet colonized with other significant organisms.

References

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3. Dasenbrook, E. et al. Persistent Methicillin-Resistant *Staphylococcus aureus* and rate of FEV_1 Decline in Cystic Fibrosis. *American Journal of Respiratory Critical Care Medicine*. 2008; 178: 814-821.
4. Ren, C. et al. Presence of Methicillin Resistant *Staphylococcus aureus* in Respiratory cultures from Cystic Fibrosis Patients is Associated with Lower Lung Function. *Pediatric Pulmonology,* 2007; 42: 513-518.
5. Cox, DW. et al. The Impact of MRSA Infection in the Airways of Children with Cystic Fibrosis: a Case-Control Study. *Irish Medical Journal*. Nov-Dec 2011; 104(10): 305-308.