Trends in incidence and susceptibility among methicillin-resistant *Staphylococcus aureus* isolated from intranasal cultures associated with rhinosinusitis

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ABSTRACT

Background: Reports regarding the incidence and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) in rhinosinusitis (RS) are limited. This study was designed to identify epidemiology and trends of MRSA incidence and antimicrobial resistance in the sinonasal cavities.

Methods: This is a retrospective case series. All intranasal/sinus cultures obtained by otolaryngologists at Stanford over a 20-year period (1990–2010) were retrospectively reviewed by mining the microbiology database. Nested searches were then made for all *S. aureus* and MRSA cultures. Patterns of incidence and changes in antibiotic susceptibilities were tabulated and statistical analysis was performed.

Results: Our search retrieved 10,387 positive intranasal culture samples, with *S. aureus* found in 800 (7.7%), and MRSA comprising 110 (1.06%) of this subset. Between the years of 1990 and 1999, only 2/112 (1.7%) of *S. aureus*-positive nasal cultures were positive for MRSA, with a sharp rise in incidence to 86/606 (14.2%) from 2000 to 2005, and to 22/82, 26.8% from 2006 to 2010. On a percent basis, using logistic regression modeling, this represents a statistically significant increasing trend ($p < 0.0001$) for MRSA sinusitis. However, over the 20-year interval studied, the patterns of antibiotic resistance among MRSA remained unaltered, especially with regard to trimethoprim-sulfamethoxazole and vancomycin.

Conclusion: *S. aureus* and MRSA isolates from intranasal cultures, which were essentially absent before the year 2000, became significantly more common earlier this decade. These data show the increased role of MRSA in sinusitis. MRSA antibiotic susceptibilities have remained, however, largely stable during this time period.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasingly appreciated pathogen that is involved in a wide range of community-acquired and nosocomial infections.¹ The first report of this resistant organism was in 1961,² shortly after the introduction of methicillin, and MRSA outbreaks became quite prevalent soon thereafter in the same decade.³ In the United States, MRSA was first isolated at Boston City hospital in 1968,⁴ and within 20 years was considered endemic in all 50 states.⁵

To receive a diagnosis of MRSA, strains of *S. aureus* typically possess a minimum inhibitory concentration to oxacillin of ≥ 4 $\mu\text{g/mL}$ using traditional testing methods. Isolates resistant to oxacillin or methicillin are usually found to be resistant to many of the antibiotics containing β -lactam rings, such as nafcillin and cefazolin.^{6,7} Currently, presence of the *mecA* gene assists in the final identification of *S. aureus* as MRSA.⁸

There has been a steady interval increase in the incidence of MRSA in both the community and the hospital settings. The percentage of MRSA among all *S. aureus* isolates from hospitals rose from 2.4% in 1975 to 29% in 1991, and to 40% in 1996.^{9,10} The increasing prevalence and involvement of MRSA in nosocomial infections highlights the importance of understanding the role of this microbe in various diseases.

Rhinosinusitis (RS) afflicts millions of patients annually, with varying degrees of severity and differing contributions of intranasal inflammation versus infection that fuels the disease process.¹¹ Numerous studies have shown that sinusitis can significantly impact overall

quality of life, in comparison with chronic debilitating diseases such as diabetes and congestive heart failure.¹² Signs and symptoms required to diagnose RS include mucopurulent discharge from the middle meatus or ethmoid region, nasal obstruction, facial pressure, and hyposmia/anosmia.¹³

Common bacterial strains isolated from patients with RS include aerobic and facultatively anaerobic Gram-positive cocci and Gram-negative species and anaerobes.^{14,15} Cultures obtained through trans-nasal, endoscopic swabs of the ethmoid cavity or middle meatus have been shown to be effective for identifying involved bacterial flora, which then leads to more accurate culture-directed therapy.¹⁶

Nadel *et al.* (1998) found a prevalence of 27% of Gram-negative rods and a 16% prevalence of *Pseudomonas* spp. in patients with chronic RS (CRS).¹⁷ Similar findings were reported by Bolger.¹⁸ In other reports on the microbiology of recurrent rhinosinusitis after endoscopic sinus surgery, Gram-positive cocci predominated (37.9%), followed by Gram-negative rods (14.8%), and in 30% of the cases, no growth was observed.¹⁹ These studies also identified a high incidence of organisms producing β -lactamase enzymes and showing other resistance to other antimicrobials, especially among coagulase-negative staphylococci.

There are limited reports regarding the prevalence of MRSA in the setting of CRS within the nasal cavity. Incidence of MRSA causing CRS was 9.22% in 2001–2003 according to Manarey *et al.*²⁰ Jiang *et al.* reported a 4.75% incidence of intranasal MRSA infection in the setting of functional endoscopic sinus surgery with CRS.²¹ Huang *et al.* analyzed the incidence of MRSA infection in acute RS in pediatric and adult cohorts.²² The incidence of MRSA was found to be 2.7%, with the most important predisposing risk factors being previous antibiotic use in children and sinus procedures in adults. This group also suggested that successful treatment of community-acquired MRSA was readily achieved with oral antibiotics.²²

Similarly, when MRSA sinusitis has been encountered, previous reports suggest a 92% rate of resolution when treated with culture-directed oral and topical medications. Oral antibiotic choices are guided by susceptibility data, but typically include trimethoprim/sulfamethoxazole or tetracyclines.²³

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Table 1 Breakdown of all positive nasal cultures from year 1990 to 2010 according to pathogen

Year	Total Positive Cultures	MSSA	MRSA	<i>Streptococcus</i> spp.	<i>Pseudomonas</i> spp.	<i>Haemophilus influenza</i>
1990–1999	1680	110	2 (0.012%)	126	112	34
2000–2005	8000	520	86 (1%)	1253	338	145
2006–2010	707	60	22 (3%)	121	52	18

MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-sensitive *S. aureus*.

The current study was undertaken to identify trends of MRSA incidence and antimicrobial resistance in nasal cavity cultures in our hospital database taken by otolaryngologists, largely representing patients with acute or CRS at a single tertiary care institution in a major metropolitan area. Furthermore, we assessed antibiotic resistance patterns for MRSA⁺ cultures, especially with regard to trimethoprim-sulfamethoxazole and vancomycin.

MATERIALS AND METHODS

After obtaining Stanford Institutional Review Board approval (no. 24947), we retrospectively reviewed the microbiological database of all nasal cavity cultures obtained at Stanford University Medical Center from January 1990 to May 2010. We limited our data mining and analysis to positive intranasal cultures (those positive for growth of an identifiable organism) that were submitted by Stanford otolaryngologists either during clinic or intraoperatively. This latter parameter was used as an indirect means of establishing an increased likelihood that positive intranasal cultures were derived from the middle meatus or other intranasal mucosal sites rather than anterior nares or other superficial nasal skin.

Laboratory Processing of Sinus Samples for Microbiological Culture

Aspirate samples were received in a Lukens trap or in an anaerobic transport vial (Anaerobe Systems, Morgan Hill, CA) if anaerobic cultures were ordered. Per standard microbiology laboratory protocols, purulent material was transferred with a swab for inoculation onto trypticase soy agar 5% sheep blood, MacConkey, and Chocolate agar plates (BD Microbiology Systems, Sparks, MD) for aerobic cultures with the addition of three anaerobic agar plates (Brucella blood with vitamin K and hemin, phenylethyl alcohol blood, and a laked kanamycin-vancomycin and *Bacteroides* bile esculin biplate) and a chopped meat carbohydrate enrichment broth (all from Anaerobe Systems) for anaerobic cultures. Aspirate material was spread thinly on a glass slide with sterile wooden sticks for the Gram stain. If the sample for anaerobic culture appeared to be tissue, it was ground up in Eugon broth (laboratory prepared) before inoculating to the media described and preparing the slide. Media were incubated in the appropriate atmosphere (5% CO₂, anaerobic boxes and chamber, or ambient air) at 35°C according to the laboratory's standard protocols. Only if no growth occurred on anaerobic plates was the chopped meat broth subcultured to media based on organisms seen in a Gram stain from the cloudy broth. Swabs were processed for aerobic culture only by being rolled directly onto the three aerobic agar plates and incubated as described previously.

Identification and Determination of Methicillin-Resistance of Staphylococci

Colonies suggestive of staphylococci on any medium (entire, convex, opaque, color white to yellow, and usually β -hemolytic on blood agar) were examined by Gram stain and 3% H₂O₂ (catalase reagent). Gram-positive cocci in clusters with a positive catalase reaction were further tested using slide or tube coagulase (rabbit plasma with EDTA). If coagulase positive, they were identified as *Staphylococcus aureus*. Antimicrobial susceptibility tests were performed using either

Figure 1. Absolute incidence and logistic regression of methicillin-resistant *Staphylococcus aureus* (MRSA) incidence over 20 years. (A) Frequency of positive nasal cultures of methicillin-sensitive *S. aureus* (MSSA), MRSA, *Streptococcus* spp., *Pseudomonas* spp., and *Haemophilus influenza* (y-axis, denoted the number of cases) (B) Trends in positive, intranasal MRSA culture on a percent basis. Error bars denote 95% confidence interval for each year's proportion, the dashed line marks the calculated trend in incidence using logistic regression analysis, and the dotted line marks the quadratic.

the MicroScan WalkAway (Siemens, Sacramento, CA), or by disk diffusion. Methicillin resistance was confirmed by cefoxitin disk during part of the study period before November 2008 and later by detection of the *mecA* gene using a laboratory-derived polymerase chain reaction test.⁸ Within the past 20 years the introduction of

Table 2 Antibiotic sensitivity trends of MRSA over time

Year/Abx	Clindamycin	Bactrim	Vancomycin	Moxifloxacin	Gentamycin	Linezolid	Rifampicin
1990–1999	1/2 (50%)	2/2 (100%)	2/2 (100%)	ND	2/2 (100%)	2/2 (100%)	2/2 (100%)
2000–2005	31/86 (36%)	79/86 (92%)	86/86 (100%)	37/86 (43%)	78/86 (91%)	84/86 (98%)	78/86 (91%)
2006–2010	11/22 (50%)	19/22 (86%)	22/22 (100%)	15/22 (68%)	19/22 (86%)	22/22 (100%)	22/22 (100%)

( ) = Denotes percent of cultures that are sensitive to a given antibiotic; ND = not determined; Abx = antibiotic; MRSA = methicillin-resistant *S. aureus*.

polymerase chain reaction in 2008 has only improved the turnaround time of drug susceptibility testing but it has not changed the sensitivity and specificity of MRSA detection.

Statistical Analysis

For each year, the number of *S. aureus* samples and the number of MRSA samples was extracted. Yearly proportions were calculated and exact (Clopper-Pearson) confidence intervals were calculated. A secular trend in the proportion of MRSA was evaluated using logistic regression, with year entered linearly (on the logit scale). The possibility of a local maximum was tested using a quadratic term for year. Logistic regression was used to determine the presence of a time trend. A quadratic was also used to allow for a possible decrease from the peak, which was only marginally significant ($p < 0.03$) using the likelihood ratio test.

RESULTS

Reviewing of the microbiology database using these parameters ultimately identified a total of 10,387 positive intranasal cultures performed at Stanford University; 6278 of the cultures were taken from women and 4109 from men. Average age of patients was 51 years.

Eight hundred (7.7%) positive intranasal cultures grew *S. aureus*, with MRSA comprising only 110 (1.06%) of this cohort. When compared with more commonly associated respiratory pathogens such as pneumococcus, *Haemophilus influenzae*, and *Pseudomonas* spp., *S. aureus* and MRSA comprised a distinct minority of cultures, as expected (see Table 1).

For trend analysis as a function of time, we then clustered our search results into three groups of years: 1990–1999, 2000–2005, and 2006–2010. When grouped into these arbitrary subsets, trends emerged. The earliest positive MRSA intranasal culture at our tertiary care institution occurred in 1998, despite the endemic nature of this pathogen in the United States at earlier time points (see beginning of article). In fact, only two positive MRSA cultures were isolated before the year 2000 (Table 1). The absolute number of MRSA⁺ cultures peaked between the years 2000 and 2005 and declined in the last cluster of years. This initially suggested a promising decline in MRSA incidence (Fig. 1 A).

However, when these same data were analyzed on a percent basis, and were subjected to logistic regression analysis, there was a statistically significant trend toward increasing incidence of MRSA ($p < 0.0001$) over time (Fig. 1 B), indicating that the proportion of MRSA in patients having RS is definitively increasing with time.

We next analyzed antimicrobial resistance patterns that have emerged over this 20-year interval to MRSA. We found that MRSA was variably but consistently resistant to clindamycin, erythromycin, and moxifloxacin over each time interval assessed. Conversely, we saw little evidence for emerging resistance of MRSA to trimethoprim-sulfamethoxazole or vancomycin, two of the current mainstay agents for MRSA treatment (Table 2). Of note, susceptibility to trimethoprim-sulfamethoxazole since the year 2000 was preserved in 86–92% of specimens, while 100% susceptibility to vancomycin was maintained throughout the 20-year interval under study.

DISCUSSION

We have been interested in determining the factors that lead to microbial growth, antibiotic resistance, and host defense in various regional subsites. In this report, we began a comprehensive evaluation of MRSA presence, and trends of antibiotic resistance, within the nasal cavity and paranasal sinuses, given the ubiquitous nature of this organism, and concerns over the future advent of “vancomycin-resistant” *S. aureus* strains.

Our findings from our mining of the microbiology database at Stanford University over a 20-year period indicate that (1) on a percent basis over time, the incidence of sinus-associated MRSA is rising; (2) there is no evidence of new antibiotic resistance patterns for intranasal MRSA in patients served in tertiary care institutions available in the literature. Isolated MRSA strains had preserved susceptibility to both trimethoprim-sulfamethoxazole and vancomycin, despite detection of this organism at our institution since at least 1998.

One of the limitations to our study is lack of data available to us regarding the intranasal subsites of the cultures, the clinical diagnoses of the patients, and the setting in which these cultures were taken. Nonetheless, we have reason to believe that the majority of our data reflect specimens obtained from patients presenting with RS, given that we studied only those intranasal culture specimens submitted by known otolaryngologists at our institution. In this setting, our experience is that cultures are most commonly taken endoscopically from the middle meatus/sinus cavities in cases of RS either in the outpatient setting or during sinus surgeries and thus most probably represent community-acquired infections. It has previously been shown that reliable, endoscopic middle meatal cultures provide a valid and meaningful culture result representing sinus cavity bacterial pathogens.^{24,25} More so, to further strengthen our assessment, the percent of MRSA in routine nares surveillance cultures at Stanford is 4.88% (NB and EJB, unpublished data, 2012), which is in contrast to the much lower 1% incidence in our subgroup analysis. This data illustrate that our cultures are not mere MRSA contamination from the anterior nares, and that there is a low likelihood that we are incorrectly ascribing a causal relationship of these cultures to RS cultures. We thus believe that our findings show a steadily increasing trend toward MRSA RS. As mentioned previously, data whether these cultures were obtained from patients with chronic versus acute RS were not available to us. Nonetheless, based on our university’s typical tertiary practice pattern and referral base, the majority of these cultures were likely to be obtained in the patients with CRS.

The root cause for the rising prevalence of this endemic pathogen is perhaps worth exploring. There is mounting evidence that MRSA colonization is associated with antecedent antibiotic use and smoking.²⁶ A recent meta-analysis suggests a direct correlation between antibiotic exposure and subsequent development of MRSA infection.²⁷ This team estimated MRSA colonization within ~120 days of prior antibiotic use, with the relative risk of acquiring MRSA elevated 1.8-fold in those patients who had taken antecedent antibiotics. Similarly, other groups report a strong association with use of fluoroquinolones and macrolides and subsequent detection of intranasal MRSA. Whether this is creating a “conducive environment” for MRSA growth, or permitting “primary selection” for resistant organisms is unclear.^{23,28}

In addition, a survey of treatment patterns by United States otolaryngologists for CRS reported use of oral antibiotics (94%) in part of their maximal medical management, with a mean duration of treatment of 5 weeks.²⁹

Finally, our results suggest that vancomycin and trimethoprim-sulfamethoxazole have remained reliable effective agents for treatment of the majority of sinonasal MRSA infections. Although mupirocin irrigation for effective treatment of MRSA sinusitis has been reported by others,³⁰ susceptibility testing to this topical agent is not currently available at our institution.

CONCLUSIONS

Longitudinal prospective studies and regular sampling for MRSA in patients with acute and chronic sinusitis are both recommended based on these data. The results of our study, which show the increased role of MRSA in sinusitis, underline the importance of performing routine endoscopic cultures, especially in patients who fail to respond to empiric antimicrobial therapy. Nonetheless, our results suggest that vancomycin and trimethoprim-sulfamethoxazole have remained reliable effective agents for treatment of the majority of sinonasal MRSA infections. Future studies include prospective stratification of MRSA sinusitis into acute community-acquired MRSA sinusitis versus chronic community-acquired MRSA sinusitis versus nosocomial MRSA sinusitis, which is difficult to accurately determine with our current database search limitations. In addition, assessing partial versus complete clinical responses to treatment with appropriate antimicrobial agents would be invaluable adjunct information to the susceptibilities as we have reported here.

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