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Concordance of nasal and diabetic foot ulcer staphylococcal colonization

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Abstract

Background—Nasal carriage of Staphylococcus aureus (SA) is an important risk factor for surgical site infections. The goal of this study was to investigate the concordance between nasal and diabetic foot ulcer (DFU) SA carriage.

Methods—79 subjects with DFUs were assessed for nasal and DFU colonization with SA, including Methicillin-resistant-SA (MRSA).

Results—Twenty-five (31.6%) subjects had nares colonization with SA; 29 (36.7%) had DFU colonization with SA. Seven (8.8%) subjects had nares colonization with MRSA and 7 (8.8%) had DFU colonization with MRSA. Ulcer duration was associated with MRSA presence (p=0.01). Sensitivity and specificity of positive nasal SA colonization with positive DFU colonization were 41 and 74%.

Conclusions—We found substantial discordance between SA strains colonizing DFU and the nasal cavity. The poor positive predictive values for SA isolation in a DFU based on nasal carriage suggests SA colonization of a DFU by endogenous SA strains cannot be assumed.

Keywords

diabetic foot; staphylococcal colonization; foot ulcer

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The results of this study have been presented, in part, at the Great Plains Emerging Infectious Diseases conference in Iowa City, Iowa and at the Wound Healing Society (WHS) conference in Denver, CO. As part of the WHS presentation, an abstract was published in *Wound Repair and Regeneration* (Volume 21, Issue 2, March-April 2013, pg. A25).

INTRODUCTION

Foot ulcers are an inevitable consequence of the metabolic, vascular and neuropathic abnormalities associated with diabetes. Ulcer healing in a person with diabetes is a complex process impacted by the above factors and the burden and nature of micro-organisms that populate chronic wounds (Falanga, 2005). This translates to serious patient morbidity, including frequent hospitalizations, repeated antimicrobial use, limb amputations and major loss of productivity. The most recent data from the US Centers for Disease Control and Prevention (CDC) show that the annual number of hospitalizations for diabetic foot “ulcer/infection/inflammation” continued to rise steadily from 1980 to 2003, when it exceeded 111,000 (Centers for Disease Control and Prevention, 2010).

Studies suggest diabetic foot ulcers (DFU) are an independent risk factor for mortality (Armstrong, Wrobel, & Robbins, 2007; Moulik, Mtonga, & Gill, 2003), and are linked directly to underlying peripheral arterial disease (PAD) and other co-existing conditions. Infection plays a major role among factors that influence DFU progression to osteomyelitis and amputation. The microbial milieu in DFU is generally polymicrobial, however *Staphylococcus aureus* (SA) is the most commonly isolated organism from both clinically infected and uninfected ulcers (Bowler, Duerden, & Armstrong, 2001; Diamantopoulos et al., 1998). Whether SA is a primary pathogen or simply a colonizer in a chronic wound is often difficult to determine. Some studies suggest growth of methicillin-resistant *Staphylococcus aureus* (MRSA) from DFU may impede wound healing time and increase likelihood of treatment failure and the need for surgical procedures, including amputation (Eleftheriadou, Tentolouris, Argiana, Jude, & Boulton, 2010; Tentolouris et al., 2006; Yates et al., 2009).

Nasal carriage of SA has been identified in several studies as one of the most important risk factors for nosocomial and surgical site infections (Bode et al., 2010; Kalmeijer et al., 2002; Perl et al., 2002; Weinstein, 1959). In cross-sectional studies, about 30% of healthy adults are found to be colonized with the organism (Kluytmans, van Belkum, & Verbrugh, 1997) and most colonized patients who become infected with SA (> 75%) are infected with endogenous strains (Bode et al., 2010; Perl et al., 2002; Weinstein, 1959). To date, only a handful of studies have explored an association between nasal SA carriage and the probability of isolating SA from DFUs (Gjodsbol, Skindersoe, Skov, & Krogfelt, 2013; Hill, Bates, Foster, & Edmonds, 2003; Stanaway, Johnson, Moulik, & Gill, 2007). Results are inconsistent, either due to small study samples or lack of strain typing amongst strains isolated from nares and DFU.

In this study, we report the prevalence of SA in DFUs and the anterior nares in an outpatient cohort of 79 subjects with non-ischemic, neuropathic DFUs that did not have clinical signs or symptoms of infection. We investigated concordance between nasal and DFU SA carriage to ascertain whether nasal screening of SA could reliably predict SA isolation from DFUs. If nasal and un-infected ulcer SA concordance is established, this knowledge will help in designing studies to identify patients with DFU at risk for infection from endogenous SA strains, and to investigate whether screening for nasal SA carriage, followed by decolonization of SA, may have a role in preventing progression of a DFU to DFI.

MATERIALS AND METHODS

Design

This study employed a cross-sectional design. Subjects with DFUs were assessed for both nasal and DFU colonization with SA, including MRSA. Patient and ulcer characteristics were concurrently measured. All study protocols were approved by the University of Iowa Institutional Review Board.

Setting and Sample

Data were collected at University of Iowa and the University of Iowa Hospitals and Clinics (UIHC). Potential subjects were recruited for screening using 1) mass media advertising, 2) clinician referrals, and 3) mailing lists of individuals who had DFUs in the past few years. Subjects were enrolled using the following criteria: 1) 18 years of age or older, 2) presence of a plantar neuropathic DFU, 3) free of systemic antibiotics over the past 2 weeks, 4) negative for clinical signs of infection, and 5) no signs or symptoms of osteomyelitis. Eligible subjects who signed a written informed consent were enrolled. Subjects with more than one DFU had one ulcer selected as the “study” ulcer based on the larger of the two ulcers. Measurement of clinical factors occurred during or immediately after screening and enrollment by a trained member of the research team. Wound and nasal specimens were also collected at this time.

Clinical Factors

Patient-level factors that were measured included age, sex, race/ethnicity, education, occupation, blood pressure, smoking history, body mass index, duration of diabetes, level of glycemic control and systemic inflammatory status. Ulcer-level factors that were measured included ulcer duration, ulcer surface area, ulcer depth, and wound tissue oxygen. Detailed protocols for measuring these variables are published elsewhere (Gardner et al., 2012; Gardner, Frantz, & Saltzman, 2005; Gardner et al., 2006).

Age, Sex, and Race/Ethnicity were measured using standard NIH definitions. *Blood pressure* was measured with cuff and stethoscope. *Smoking history* was measured as self-reported packs/day, years/smoked, and pack years. *Body mass index* was measured as weight(kg)/height(m)^2. *Duration of diabetes* was measured as the number of years since diagnosis based on subject report. *Systemic inflammatory status* was measured as plasma C reactive protein and whole blood erythrocyte sedimentation rate levels. *Level of Glycemic Control* was measured as hemoglobin A1c values (HbgA1c). The presence of retinopathy, end stage renal disease (ESRD) and chronic renal insufficiency were extracted from the medical record. Level of education and occupation were extracted from the clinical database maintained on all patients.

Duration of the study ulcer was measured as number of weeks from soft tissue loss (i.e., epidermis, dermis, etc.) based on subject report and review of medical records. Ulcer size, including *surface area and depth*, was measured using digital images and proprietary software (Gardner et al., 2012). *Wound Tissue Oxygen* was measured using transcutaneous

oxygen measures ($tcpO_2$) (Radiometer America, Inc., Model TCM400, Denmark) on the dorsum of the ipsilateral foot.

Nasal and Ulcer Cultures

Specimens from nares were obtained by inserting a LQ Stuart Culture Swab (BBL) approximately 2 cm into the nares and rotating the swab against the anterior nasal mucosa for 3 seconds. This process was repeated in the other nare. Specimens of ulcer microbes were obtained using Levine's technique using established study protocols (Gardner et al., 2006). Levine's technique is different than other swab specimen techniques in that it samples fluid from deep tissue layers. The wound was cleansed with non-bacteriostatic saline and an Amies with charcoal transport swab (Copan, Italy) was rotated over a 1-cm^2 area of viable, non-necrotic wound tissue for five seconds using sufficient pressure to extract wound tissue fluid. We found Levine's technique to have an accuracy (Area Under the ROC Curve) of 0.80 when compared to wound tissue specimens, the gold standard (Gardner et al., 2006). Both the nasal and ulcer swabs were immediately transported to a dedicated microbiological research laboratory.

Nasal swabs were subcultured onto Columbia blood agar (Remel, Lenexa, KS), and CHROMagar MRSA™ (BD, Sparks, MD). DFU swabs were plated on Columbia blood agar, eosin-methylene blue agar (EMB; Remel, Lenexa, KS) and CHROMagarMRSA™. Columbia and EMB plates were incubated in 5% CO_2 at 37°C for 48 hours, and MRSA plates were incubated aerobically at 37°C for 48 hours. Additionally, swabs were plated on Brucella Agar supplemented with blood, hemin and Vitamin K (Remel, Lenexa, KS) and incubated in an anaerobe jar at 37°C for 48 hours. The plates were examined for growth and colony characteristics. Mauve colored colonies on the CHROMagarMRSA plates are indicative of MRSA and were subcultured for identification. Differing colony morphologies on the Columbia and EMB plates were subcultured for identification. All organisms isolated were identified to the species level using standard microbiological procedures (Murray, Baron, Pfaller, Tenover, & Yolkin, 1999).

Antimicrobial susceptibility testing was performed on all organisms identified as SA using the Clinical Laboratory Standards Institute (CLSI) broth microdilution method (Clinical and Laboratory Standards Institute, 2012a, 2012b). Genetic relatedness of SA was determined using PFGE according to published methods (Pfaller, Caliendo, & Versalovic, 2010). Chromosomal DNA was digested using SmaI (Sigma-Aldrich, St. Louis, MO) and separated using a CHEF DR II machine (BioRad, Hercules, CA). Gel patterns were analyzed using Bionumerics software (Applied Maths, Kortrijk, Belgium). Patterns were compared to type strains USA100 – USA1200 (McDougal et al., 2003). All SA isolates were screened by PCR for the mecA gene according to previously published methods (Mendes et al., 2007; Richter et al., 2011).

Data Analysis

For descriptive statistics, counts and percentages were reported for discrete variables, while means and standard deviations were reported for continuous variables. Medians and interquartile ranges were reported when sample sizes were small. Univariate analysis

compared patient factors and ulcer characteristics between patients with SA positive and negative cultures, from both nares and ulcer swabs. Comparisons were also made this way for patients with positive or negative MRSA swabs, given they were positive for SA at that site. This was done for discrete variables using the chisquared test, for continuous variables using the student's t-tests and the Fisher's exact test or nonparametric Wilcoxon–Mann–Whitney rank sum test were used when sample sizes were small. Univariate logistic regression was also used to examine the relationship between patient/ulcer factors and a positive SA ulcer.

The association between a positive SA nare culture and a positive SA DFU was further assessed using logistic regression while controlling for potential confounders. All patient factors and ulcer characteristics were individually screened for confounding and interaction. Patient factors that meaningfully changed the exposure odds ratio (OR) were included in the final logistic regression model. All statistical analyses were conducted using SAS software (version 9.3; SAS Institute, Cary, NC), with significance set at a P value < 0.05.

RESULTS

Seventy-nine subjects completed the study. Patient and ulcer characteristics are presented in Tables 1 and 2. Twenty-five (31.6%) subjects were positive for nares colonization with SA, while 29 (36.7%) subjects were positive for DFU colonization with SA (see Tables 1 and 2). The number of SA strains per colonized nares or ulcer ranged from 1 to 3 with a mean of 1.12 (SD = .44) and 1.17 (SD = .47), respectively. Because of missing data and small number of subjects per cell, race/ethnicity, occupation, and education were not examined for their association with SA colonization. None of the factors analyzed were associated with positive SA ulcer colonization. Odds ratios for each patient and ulcer factor are presented in Tables 1 and 2.

Seven (8.8%) subjects were positive for nares colonization with MRSA and seven (8.8%) subjects were positive for DFU colonization with MRSA (see Tables 1 and 2). Longer duration of the ulcer was positively associated with the presence of MRSA in the ulcer (t-test: p=0.01). The sensitivity, specificity, positive predictive value, and negative predictive value of positive SA and MRSA nasal colonization with positive DFU colonization are shown in Table 3.

A positive SA nares culture was not significantly associated with a positive DFU culture (Odds ratio = 2.01, CI (0.76–5.312), p=0.16), however, when controlling for identified confounders there was a significant association. BMI, smoking pack-years, duration of diabetes and mean tissue oxygen were determined to be confounders as described above and were included in the final logistic regression model as control variables. Controlling for confounders, subjects with a positive SA DFU culture had an over 4-fold increased odds of having a positive SA nares culture (Odds ratio = 4.16, 95% CI (1.27 – 13.61), p=0.02).

Among subjects with both nasal and ulcer SA (n=12), PFGE analyses showed 58% concordance (7/12 patients) with strain-relatedness of SA. Among the subjects with both

nasal and ulcer MRSA (n=3), PFGE analyses showed 33% concordance (1/3 patients) with strain-relatedness of MRSA.

DISCUSSION

With the increasing rate of SA infections in the community and healthcare settings, it is important to uncover potential reservoirs of the bacteria. Several studies identified nasal staphylococcal carriage as one of the most important sources of SA strains causing infection (von Eiff, Becker, Machka, Stammer, & Peters, 2001; Wenzel & Edmond, 2001; H. F. L. Wertheim et al., 2004). Screening for, and elimination of, nasal SA carriage is therefore an attractive method to decrease SA infections. We hypothesized a significant concordance between nasal and foot ulcer SA strains in persons with diabetes. Such an association could be used to design trials examining new approaches to manage and/or prevent SA infectious complications of DFUs.

High rates of SA colonization are reported for certain patient groups. These include hospitalized patients, insulin and non-insulin dependent persons with diabetes, patients on hemodialysis therapy for end-stage renal disease, patients with HIV infection or chronic liver disease, individuals with obesity, SA skin infections or chronic skin disorders and intravenous drug abusers and prison inmates (H. F. Wertheim et al., 2005). Large population-based studies have estimated the rate of SA adult nasal carriage in the United States at around 29% (Gorwitz, 2008).

A recent meta-analysis reviewed preoperative SA nasal decolonization in cardiac and orthopedic patients (Schweizer et al., 2013). The study provided evidence that decolonization and modification of surgical prophylaxis based on SA nasal carriage was protective against staphylococcal surgical site infections. Several studies have also demonstrated that eradication of nasal SA carriage in patients undergoing hemodialysis reduces infection (H. F. Wertheim et al., 2005). It is unclear if a similar infection prevention approach could be applied to an ambulatory patient population, such as people with diabetes and un-infected foot ulcers.

To date, only 3 clinical studies have explored an association between nasal SA and chronic ulcer colonization in ambulatory populations. Two of these studies evaluated DFUs (Hill et al., 2003; Stanaway et al., 2007). One study (n=65) identified 44 bacterial isolates in DFUs, of which 27 were SA (42%) (Stanaway et al., 2007); MRSA comprised 12/27 of the SA ulcer isolates (44%). The total number of nasal SA strains isolated was not specified. MRSA was isolated from the nares of 11/65 total subjects (17%). Concordance between nasal and ulcer MRSA was 58% (7/12) and the OR for isolating MRSA from a DFU in a MRSA nasal carrier was 17.2 (95% CI: 3.7–79.6). Importantly, ulcer and nasal SA strain typing was not performed. The other DFU study (Hill et al., 2003) included genotypic analysis of nasal and DFU SA strains and demonstrated a 92% concordance. The third study evaluating nasal and ulcer SA concordance focused on chronic venous leg ulcers (Gjodsbol et al., 2013). In this study, SA was isolated from 13/16 ulcers (83%). Six of the 13 patients also harbored SA in the nasal cavity (8%). No MRSA strains were isolated. Pulse field gel electrophoresis (PFGE) analysis of nasal and ulcer SA strains demonstrated 100% concordance. This could

be due to cross-contamination of the ulcer by endogenous SA strains or to cross-contamination of the nares by ulcer SA strains. There were significant differences between the methodologies of these 3 studies. The two DFU studies included all ulcer types and did not specify if these ulcers were infection-free upon enrollment or been previously treated.

Our work adds to the current knowledge of the epidemiology of uninfected DFU and nasal SA colonization. In our cohort of 79 DFUs, 29 subjects (36%) were colonized with SA in the ulcer. MRSA comprised 7/29 (24%) of the SA ulcer strains. Twelve of the 29 subjects (41%) had SA in both the nares and DFU. Of these 12 subjects, 3 had MRSA (25%) in both the nares and ulcer. Of those with SA carriage in the nares and ulcer, PFGE analyses showed 58% (7/12) concordance in strain-relatedness of SA. Of those with MRSA carriage in the both nares and ulcer, only 1/3rd had concordant MRSA strains by PFGE analysis.

These findings may have important clinical implications. Our study finds substantial discordance between SA strains colonizing DFU and the nasal cavity. Moreover, the poor positive predictive values (43–48%) for SA and MRSA isolation in a DFU based on nasal carriage implies that, at least in the ambulatory setting of an un-infected DFU, SA colonization of an ulcer by endogenous SA/ MRSA strains cannot be assumed to occur. From a therapeutic standpoint, in the absence of wound information, nasal SA screening results could potentially mislead clinicians in antimicrobial selection of a DFU infection. Therefore, screening for and decolonization of SA nasal carriage in a patient with a DFU may not be a useful approach to prevent a diabetic foot infection *if viewed in isolation*.

Any relationship between nasal and ulcer SA colonization is confounded by other patient factors, such as BMI, smoking pack-years, duration of diabetes and mean tissue oxygen. Similarly, the local environment of a DFU and the systemic factors that govern its development also influence bacteria that colonize the ulcer. Factors unique to chronic wounds, such as ulcer surface area, depth, grade, poor vascular supply or glycemic control may allow for MRSA persistence in a DFU. However, none of these factors reached statistical significance in our study, but this may be due to the small number of study ulcers with MRSA. An important factor that contributes to delayed wound healing is the wound microbiome. Chronic wounds have been shown to harbor a diverse microbial milieu (Gardner, Hillis, Heilmann, Segre, & Grice, 2013) that survives by complex intra- and inter-species interactions. In a polymicrobial environment such as a chronic DFU, the persistence of MRSA can therefore, be attributed to both its virulence factors as well as to a synergistic effect of neighboring bacteria. Hence, infection prevention strategies that have been successful in reducing SA surgical site infections or invasive infections in ICU and hemodialysis patients may not be easily extrapolated to this patient population.

In our study, ulcer duration was the only patient related factor that was significantly associated with MRSA colonization; ulcers of longer duration were more likely to be MRSA colonized. This finding could have several possible explanations. Patients with chronic DFUs are more likely to have healthcare exposure and hence, increased probability of MRSA colonization. Patients with a DFU, whether infected or un-infected are frequently prescribed antimicrobial therapy; repeated antimicrobial exposure is well-known to promote MRSA colonization. Only a few of the DFUs in our study had prior antibiotic exposure

before being enrolled and none had antibiotic exposure in the 2 weeks prior to data collection.

A limitation of our study was that we included only clinically uninfected and neuropathic ulcers. Infected, previously treated or ischemic DFUs may have a different microbial milieu in terms of number and diversity. This would be a result of antimicrobial pressure and levels of tissue oxygenation. Therefore, the propensity to infection in such ulcers may also differ from “clean”, neuropathic ulcers (Prompers et al., 2008). However, the aim of this study was not to evaluate an association between nasal SA carriage and an infected DFU, rather to investigate the concordance between nasal and ulcer SA strains before an infection develops. Other limitations were the small sample size and that only the anterior nares were cultured to evaluate SA colonization. Several authors evaluating benefit of staphylococcal decolonization programs (Bradley, 2007; Fritz et al., 2012) have identified extra-nasal sites of SA carriage (pharynx, axilla, perineum) as potentially important reservoirs. However, the anterior nares is believed to be the most frequent carriage site for SA (H. F. Wertheim et al., 2005). An additional limitation is that broth enrichment was not used to isolate SA from the nares specimens. Broth enrichment may have increased the recovery of SA and MRSA.

In summary, we believe our work raises interesting questions about the patterns of SA colonization and the role of endogenous SA strains in DFU microbiology. Further work is needed to confirm the findings of this study. It would be extremely useful to investigate concordance between SA strains, in particular MRSA in the nares and infected DFUs and to determine the incidence of DFI in a nasal SA carrier. This would be particularly relevant to the fact that SA cultured from a DFU is frequently, only a surface colonizer and that the significance of a positive swab culture for SA from an infected DFU is not known at present. If a significant association between nasal and DFU/DFI SA colonization is demonstrated, further studies could assess the potential utility of SA screening and decolonization strategies for infection prevention. Furthermore, the role of surface cultures (of nares and a DFU) in guiding antimicrobial therapy could be better defined.

We hope to explore these findings further in larger, prospective studies that compare the incidence of DFU infections with both SA concordant and discordant strains as well as trends in SA strains inhabiting the nares and ulcer over time.

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Table 1

Patient characteristics and odds ratios for *Staphylococcus aureus* in the diabetic ulcer (N=79)

Patient Factors			Odds Ratio (CI): Ulcer SA Positive	Chi-square P-value
Age in years, mean (SD)	55.0	±11.5	1.02(0.97–1.06)	0.3981
Male, n (%)	64	81.0%	1.20(0.37–3.93)	0.7633
Race, n (%)				
White	72	91.1 %	0.67(0.12–3.68)	0.6418
Non-white	7	08.9%		
Body Mass Index (BMI) in kg/m^2, mean (SD)	34.3	±6.6	1.04(0.96–1.12)	0.3027
Systolic Blood Pressure (SBP) in mmHg, mean (SD)	134.9	±18. 9	1.02(0.99–1.04)	0.1759
Smoking History				
Current Smoker, n (%)	49	63.6%		
Pack years, mean (SD)	25.6	±28.6	1.01 (0.99–1.03	0.4279
Duration of Diabetes in years, mean (SD)	16.0	±11.7	0.98(0.94–1.02)	0.3737
Hemoglobin (Hb) A1C % (mmol/mol), mean (SD)	8.3	±2.0	0.86(0.68–1.09)	0.2153
HbA1C<5.5, n(%)	3	3.8%		
HbA1C5.5–7.0, n (%)	24	30.4%		
HbA1C>7.0, n(%)	52	65.8%		
C Reactive Protein in mg/L, mean (SD)	2.4	±5.3	1.07 (0.92–1.12)	0.7469
Erythrocyte Sedimentation Rate in mm/hr, mean (SD)	30.8	±22.8	1.01 (0.99–1.03)	0.5509
Retinopathy, n (%)	30	42.9%	1.15 (0.43–3.05)	0.7768
Renal Disease, n (%)	16	20.3%	0.69 (0.23–2.11)	0.5142
Nares SA positive, n (%)	25	31.6%	2.01 (0.76–5.31)	0.1596
Nares MRSA positive, n (%)	7	08.8%		

SA = *Staphylococcus aureus*
MRSA = Methicillin-resistant SA

Table 2

Foot ulcer characteristics and odds ratios for *Staphylococcus aureus* in the diabetic ulcer (N=79)

Ulcer Factors			Odds Ratio (CI): Ulcer SA Positive	P-value
Duration in weeks, mean (SD)	32.8	±40.51	1.00(0.99–1.02)	0.4744
Circumference in cm, mean (SD)	4.7	±2.82	0.96(0.82–1.13)	0.6194
Surface Area in cm^2, mean (SD)	1.9	±2.56	0.97(0.81–1.15)	0.6879
Depth in cm, mean (SD)	0.4	±0.23	0.90(0.17–4.76)	0.8985
Volume in cm^3, mean (SD)	0.3	±0.45	0.78 (0.23–2.62)	0.6914
Ulcer tissue oxygen mmHg, mean (SD)	47.0	±14.30	1.03(0.99–1.064)	0.1242
Toe Brachial Pressure Index, mean (SD)	0.89	±0.25	1.68(0.26–10.89)	0.5881
Ulcer SA positive, n (%)	29	36.7%	------	
Ulcer MRSA positive, n (%)	7	08.8%		

SA = *Staphylococcus aureus*
MRSA = Methicillin-resistant SA

Table 3

Concordance between nasal and diabetic foot ulcer *Staphylococcus aureus* (SA) and Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization (N=79)

	Ulcer SA+	**Ulcer SA−**	**Total**	
Nasal SA+	12	13	25	Positive Predictive Value = 48.0
Nasal SA−	17	37	54	Negative Predictive Value = 68.5
Total	29	50	79	
	Sensitivity = 41.4	Specificity = 74.0		
	Ulcer MRSA+	Ulcer MRSA−	Total	
Nasal MRSA+	3	4	7	Positive Predictive Value = 42.9
Nasal MRSA−	4	68	72	Negative Predictive Value = 94.4
Total	7	72	79	
	Sensitivity = 42.9	Specificity = 94.4		