Outcomes of Beta-Hemolytic Streptococcal Necrotizing Skin and Soft Tissue Infections and the Impact of Clindamycin Resistance

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Abstract

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Background: Beta-hemolytic streptococci are frequently implicated in necrotizing soft tissue infections (NSTI). Clindamycin administration may improve outcomes in patients with serious streptococcal infections. However, clindamycin resistance is growing worldwide, and resistance patterns in NSTI and their impact on outcomes are unknown.

Methods: Between 2015 and 2018, NSTI patients at a quaternary referral center were followed for death, limb loss, and streptococcal toxic shock syndrome (STSS). Surgical wound cultures and resistance data were obtained within 48 hours of admission as part of routine care. Risk ratios for the association between these outcomes and the presence of beta-hemolytic streptococci, or presence of clindamycin-resistant beta-hemolytic

streptococci were calculated using log-binomial regression, controlling for age, transfer status, and injection drug use-related etiology.

Results: Of 445 NSTI identified, 85% had surgical wound cultures within 48 hours of admission. 31% grew beta-hemolytic streptococci, and clindamycin resistance was observed in 32% of patients. The presence of beta-hemolytic streptococci was associated with greater risk of amputation (RR 1.80 [95% CI 1.07-3.01]), as was the presence of clindamycin- resistance among beta-hemolytic streptococci infections (RR 1.86 [95% CI 1.10-3.16]).

Discussion: Beta-hemolytic streptococci are highly prevalent in NSTI, and in our population clindamycin resistance was more common than previously described. Greater risk of limb loss amongst patients with beta-hemolytic streptococci—particularly clindamycin-resistant strains—may portend a more locally aggressive disease process or may represent pre-existing patient characteristics that predispose to both infection and limb loss. Regardless, these findings may inform antibiotic selection and surgical management to maximize the potential for limb salvage.

INTRODUCTION

Necrotizing soft tissue infections (NSTI) represent a heterogeneous group of rapidly progressive skin and soft tissue infection. In the United States, there are approximately 3,800 to 5,800 cases annually, with modern series' reporting case-fatality between 8 and 17% as well as substantial morbidity and functional limitations.[1–4] NSTI are caused by a variety of pathogens, and can be either monomicrobial or polymicrobial in nature. Though *Clostridium perfringens* is the most feared organism implicated in NSTI due its associated high case-fatality,[5] streptococcal species are considerably more common.[6] Beta-hemolytic streptococcal species, including *Streptococcus pyogenes* (group A streptococcus, GAS), *Streptococcus agalactiae* (group B streptococcus, GBS) and *Streptococcus dysgalactiae subspecies* (SD), have all been implicated to varying degrees and in different populations.[7–13]

Penicillin is the primary treatment for beta-hemolytic streptococcal infection. The use of clindamycin is recommended for documented GAS by the Infectious Diseases Society of America (IDSA).[14] The addition of clindamycin has been associated with improved outcomes in patients with serious streptococcal infections including those with streptococcal toxic shock syndrome (STSS), and penicillin monotherapy is associated with higher morbidity and mortality compared to penicillin plus clindamycin.[15–18] Preclinical studies demonstrate that clindamycin reduces the production of bacterial toxins which mediate the intense inflammatory response.[19–22] In vivo studies using murine NSTI models have demonstrated that clindamycin leads to a reduction in virulence factor activity amongst GAS strains,[23] but this has not been well evaluated in humans.

Unfortunately, clindamycin resistance is emerging in streptococcal species worldwide.[24–26] In the US, it was encountered in less than 1% of GAS isolates in

2003;[27] this figure increased to 15% in 2015.[28] Even higher levels of resistance are reported in GBS isolates.[29,30] Between 2006-2010 in France, clindamycin resistance was higher for SD than for GAS during the same period.[31] A study in Ontario, Canada between 2011-2012 identified 14 cases of invasive SD, of which all were penicillin sensitive, but 14% were clindamycin resistant.[13]

Antibiotic resistance profiles in NSTI have not been clearly characterized, though clindamycin is strongly recommended as adjunct antibiotic regimen despite its associated risk of *Clostridioides difficile* pseudomembranous colitis. Furthermore, it is unknown whether patient outcomes differ based on clindamycin resistance. Using a large, prospective NSTI cohort, we sought to describe clindamycin resistance patterns and determine whether patient outcomes differed based on the presence of beta-hemolytic streptococci and as well as clindamycin resistant isolates. We hypothesized that the presence of beta-hemolytic streptococcal species—particularly clindamycin resistant strains—would be associated with worse outcomes.

METHODS

Data collection

All adult patients presenting with necrotizing skin and soft tissue infections at Harborview Medical Center, Seattle, WA, were included in a prospective, internal registry beginning in January of 2015. Harborview is a 413-bed acute care hospital that serves as a public safety-net hospital for King County, as well as the level I trauma and burn center for Washington, Alaska, Montana, and Idaho. Cases were confirmed by review of operative and admission records. American Association for the Surgery of Trauma Grade IV and V soft tissues infections were considered NSTI for the purposes of this study.[32] Patients

with surgical wound cultures obtained within 48 hours of admission were eligible for inclusion in this study.

Wound cultures were obtained as part of routine patient care. Clindamycin resistance of beta-hemolytic streptococcal species (GAS, GBS, or SD) was determined using standard procedures involving clindamycin- and erythromycin-impregnated disk diffusion to evaluate for resistance; inducible vs. constitutive mechanism of resistance was determined by the shape of the zone of inhibition.[33] The primary outcomes were in-hospital mortality, amputation among patients with limb involvement, and early STSS among patients with beta-hemolytic streptococcal species. The presence of STSS within 24 hours of admission was determined based on the CDC criteria: hypotension (systolic blood pressure [SBP] less than 90 mmHg) and organ failure (characterized by creatinine greater than 2 m/dL; platelets less than 100 x 10^3 / μL; aspartate aminotransferase, alanine aminotransferase, or total bilirubin greater than 2 times the upper limit of normal; or acute respiratory distress syndrome).[34] Key covariates—age, whether the patient was transferred from another facility, and whether the infection etiology was injection drug use-related—were chosen a priori.

This study was performed with the approval of the University of Washington Institutional Review Board and informed consent was waived.

Statistical analysis

Data were analyzed using R (Version 3.6.1). Categorical variables were reported as absolute values and percentages, and continuous variables as medians and interquartile ranges. Log-binomial regression was used to estimate risk ratios, controlling for potential

confounders (injection drug use, transfer status, and age). Unadjusted and adjusted risk ratios are presented with associated 95% confidence intervals.

RESULTS

Patient and infection characteristics

Between 2015 and 2018, 445 adult patients with NSTI were identified. 435 (98%) underwent at least one surgical debridement at our institution, of which 377 (87%) had intra-operative wound cultures obtained as surgical specimens within 48 hours of admission and were included in this study (Table I). Patients were predominantly middle-aged (median age 55 years, [IQR 44-62]), male (66%), and white (78%). The majority of patients were transferred from another institution (88%). Diabetes was the most common pre-existing condition. The perineum was the most frequently involved site (37%), followed by the leg (33%). Beta-hemolytic streptococcal species were common among infections involving the leg and arm. Nearly a third of infections had no clear etiology (e.g. trauma, post-operative).

Microbiology, antibiotic, and sensitivity data

Figure I provides a flowchart of patient culture data. There were 120 beta-hemolytic streptococcal isolates identified among 118 patients. Twenty-five patients (21%) with beta-hemolytic streptococci lacked susceptibility data. Among those with susceptibility data, 32% of patients (29/93) were found to have a clindamycin resistant beta-hemolytic streptococcal species. Clindamycin resistance was observed in 26% (19/73) of GAS isolates, 50% (9/18) of GBS isolates, and 67% (2/3) of SD isolates. Resistance was constitutive in 33% (10/30) of resistant isolates (2/19 GAS, 8/9 GBS and 0/2 SD isolates).

Clindamycin resistance was more common amongst patients who presented primarily to our institution (57%) compared to those who were transferred (23%). Transfer patients were 59% less likely to have a clindamycin resistant strain, controlling for injection drug use (RR: 0.41 [95% CI 0.24-0.72]).

Table II summarizes the most frequent organisms observed in wound cultures. Overall, the majority of infections were polymicrobial (73%). Of the beta-hemolytic streptococci, GAS was more likely to be found alone, whereas GBS and SD were more commonly part of a polymicrobial culture. Beta-hemolytic streptococci were most frequently co-cultured with *Staphylococcus* species: 47% were co-cultured with a *Staphylococcus* species, of which 13% were cultured with methicillin-resistant *Staphylococcus aureus* and 15% with methicillin-sensitive *Staphylococcus aureus*. Among patients with wound cultures, 5% of patients also had a positive blood culture during the same time period.

Within 12 hours of admission, 94% of patients who ultimately grew beta-hemolytic streptococci had received clindamycin, and 69% had received penicillin. At our institution, patients with suspected NSTI typically receive a high dose of clindamycin: 1200 mg IV every six hours. Two of the seven who did not receive clindamycin, and 19 of the 25 patients who did not receive penicillin, reported an allergy to that particular medication. One patient received linezolid in place of clindamycin, and 12 of the 25 patients who did not receive penicillin received an alternate beta-lactam. *Clostridioides difficile* colitis occurred in 3% of the overall NSTI cohort, 4% of patients with beta-hemolytic streptococci, and 3.4% of patients with clindamycin resistant isolates.

Treatment and clinical outcomes

Overall, the median hospital length of stay was 19 days [IQR 11, 30], though ICU stays were relatively short with few ventilator days (Table III). Amputation was required in 23% of patients with limb involvement overall, and was more common among patients with beta-hemolytic streptococci (15% vs 32%). Unadjusted and adjusted risk ratio estimates for the associations between the presence and absence of beta-hemolytic streptococci and the outcomes of interest are provided in Table IV. The presence of beta-hemolytic streptococci was associated with greater risk of amputation (adjusted RR 1.79 [95% CI 1.07-3.01]). This association did not differ depending on whether the beta-hemolytic streptococci were part of a monomicrobial or polymicrobial infection. STSS early in patients' hospital courses was rare, occurring in only 5% of patients with beta-hemolytic streptococci; however, 33% (2/6) patients with early STSS died, compared to 9% (10/112) without STSS. The overall mortality was 15% in our study cohort. No differences in mortality were observed based on presence or absence of beta hemolytic streptococci.

Unadjusted and adjusted risk ratio estimates for the associations between clindamycin resistance among patients with beta-hemolytic streptococci and the outcomes of interest are provided in Table V. In univariate analysis, there were no observed differences in amputation, STSS or mortality based on clindamycin susceptibility. In pre-specified multivariate analysis, clindamycin resistance was associated with 86% greater risk of amputation (adjusted RR 1.86 [95% CI 1.10-3.16]) compared to patients with clindamycin sensitive beta-hemolytic streptococci, but no appreciably altered risk of early STSS (adjusted RR 1.23 [95% CI 0.25-6.08]) or mortality (adjusted RR 1.38 [95% CI 0.41-4.63]) were observed.

DISCUSSION

Using one of the largest prospectively collected NSTI databases, we observed that beta-hemolytic streptococci were frequently identified in NSTI surgical wound cultures, and—for infections involving extremities—were associated with a greater risk of amputation compared to infections not involving beta-hemolytic streptococci. Clindamycin resistant streptococcal infections were also associated with a relatively greater risk of amputation compared to those involving clindamycin sensitive streptococci.

Beta-hemolytic streptococcal infections as a risk factor for amputation in NSTI has never been previously investigated. It is unclear whether beta-hemolytic streptococcal species—through rapidly progressive and extensive necrosis preventing limb salvage—are the primary determinant for amputation, or instead signify other patient conditions that both predispose to amputation (e.g. peripheral vascular disease or diabetes) and provide an ideal environment for beta-hemolytic streptococcal infection. Diabetes was particularly common in our population, and though no indicator of underlying vascular disease was available, a chronic wound was noted as the infection etiology in 16% of patients with beta-hemolytic streptococcal species. *Streptococcus* spp. are not infrequently found in chronic wounds associated with diabetes or peripheral vascular disease.[35] However, the host and/or organism characteristics which push sub-acute or chronic wounds into the realm of NSTI are not well defined.

Clindamycin resistance among beta-hemolytic streptococci was more prevalent than previously reported. One recently published study out of five Scandinavian centers focusing on GAS and SD NSTI found only 1 out of 113 streptococcal isolates were resistant to clindamycin.[7] In our cohort, resistance was particularly common among patients presenting directly to our hospital. This may be related to the fact that our institution is a

safety net for the underserved and those living homeless in our large, urban community. As such, the prevalence of clindamycin resistance reported in this study may not be generalizable to other institutions, which often have their own resistance patterns and antibiograms. However, differential frequencies of resistance observed in patients originating from rural or urban centers may be useful in guiding antibiotic selection and highlight the need for intensive surveillance of resistance patterns.

Clindamycin resistance was also associated with greater risk of amputation. Clindamycin may play an important role in minimizing the locally destructive nature of beta-hemolytic streptococcal infections, and resistance may mitigate this. Alternatively, patients with clindamycin resistant strains may have had more interaction with the health care system and this may simply be a marker of underlying medical conditions that predispose a patient to amputation. Given the risk associated with direct admission compared to being transferred in, there may also be an association with higher risk exposures to individuals or at-risk groups colonized or infected with clindamycin-resistant beta-hemolytic streptococci. The risk ratio estimate relating clindamycin resistance to amputation changed considerably after controlling for potential confounders, which were selected a priori. Transfer status in particular is likely contributing to confounding as it was noted to be associated with lower risk of clindamycin resistance in this study, and has previously been shown to be associated with greater likelihood of amputation in NSTI.[36]

Clindamycin is thought to have a bacteriostatic, and possibly bactericidal, effect in *Streptococcus* species by binding ribosomal subunits and inhibiting protein synthesis, and is believed to improve outcomes by inhibiting the production of key virulence proteins. Resistance is possible through a number of mechanisms, including modification of ribosomal targets through mutation or methylation, or increased efflux.[37] One would

expect clindamycin resistance to mitigate the benefit of clindamycin on clinical outcomes. However, because nearly every patient in this cohort received clindamycin on admission, it was impossible to assess whether the previously described benefit of adjunct clindamycin administration is negated by clindamycin resistance. This leads us to postulate whether linezolid, an oxazolidinone that inhibits bacterial protein synthesis, may reduce virulence factor and the production of toxins in clindamycin resistant isolates, and potentially improve outcomes. Although linezolid has been shown to be effective in uncomplicated skin and soft tissue infections, the clinical utility of linezolid as an alternative to clindamycin will need to be further evaluated in NSTI.

Beyond limb NSTI, the predictive value of the presence of beta-hemolytic streptococci—and clindamycin resistance in beta-hemolytic streptococci in NSTI—may be limited, as no differences in risk of death or development of STSS early in hospitalization were observed. Our study was underpowered to detect small differences in the occurrence of these outcomes, as mortality and prevalence of STSS were both low. Alternatively, this finding may reflect that beta-hemolytic streptococci play a relatively minor role in the systemic manifestations of NSTI.

We opted to utilize wound culture data rather than blood cultures for this study. Blood cultures are generally inefficient at identifying pathogen(s) responsible for infection.[38] In our cohort, few patients had positive blood culture results, and even fewer were positive for streptococci species. Culture data were only included in this analysis if the culture had been obtained within 48 hours of admission; this was intended to minimize potential for detecting colonizing—rather than disease-causing—organisms. We believe that wound culture data provide a useful insight into the pathophysiology of NSTI, as the majority of NSTI are polymicrobial, and wound cultures reflect the complex, diverse

bacterial milieu of NSTI, without necessarily definitively pinpointing the causative organism(s). The diversity of bacterial profiles that have been described in NSTI makes it difficult to draw conclusions regarding necessary and sufficient conditions for the development of NSTI; however, a secondary analysis of our data suggested that the association with amputation did not differ based on whether or not the beta-hemolytic streptococci were part of a monomicrobial or polymicrobial infection.

This study has a few limitations. Wound culture data were missing in 15% of patients, and of those with streptococcal isolates, 21% lacked resistance data. This missingness is likely related to transfer status and debridement history, as outside cultured data were unavailable, and a small fraction of patients were transferred after having been adequately debrided or died before operative intervention was possible. The timing of amputation relative to admission was not available; however, early amputation may represent more advanced disease on presentation, whereas later amputation may be more amenable to the potential benefits of clindamycin. Finally, the majority of patients were transferred from another institution. This may select for extreme cases, which require higher levels of care that are more appropriate for a quaternary referral center and may limit generalizability.

In summary, the data presented in this study suggest that among patients with extremity NSTI, knowledge of the organism involved, as well as its resistance to clindamycin, can help to predict the need for limb amputation. We also demonstrated a high degree of clindamycin resistance within our community, particularly among highly virulent, invasive *Streptococcus* species. The empiric use of clindamycin, and the potential use of alternative antimicrobial agents (e.g linezolid) in clinical settings with high prevalence of clindamycin resistance requires further evaluation. These results also

highlight the need for additional inquiry into the complex microenvironment and host-organism interactions that facilitate the development of necrotizing infections in order to identify modifiable patient or disease characteristics and additional interventions to facilitate limb salvage.

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Table I: Demographic and clinical characteristics of NSTI patients with culture data overall (n=377), and by presence (n=118) and absence (n=259) of beta-hemolytic streptococci.

		Beta-Hemolytic Streptococci	
	Total (n=377)	**Absent (n=259)**	**Present (n=118)**
Male sex, n (%)	248 (65.8)	164 (63.3)	84 (71.2)
Age (years), median [IQR]	55.0 [44.0, 62.0]	56.0 [44.5, 63.0]	53.0 [43.3, 60.8]
Race, n (%)			
White	294 (78.0)	202 (78.0)	92 (78.0)
Black	19 (5.0)	14 (5.4)	5 (4.2)
Native American	11 (2.9)	8 (3.1)	3 (2.5)
Asian	6 (1.6)	4 (1.5)	2 (1.7)
Other/Unknown	47 (12.5)	31 (12.0)	16 (13.6)
Transfer Patient, n (%)	332 (88.1)	238 (91.9)	94 (79.7)
Pre-Existing Conditions, n (%)			
CRF	26 (6.9)	23 (8.9)	3 (2.5)
Diabetes	166 (44.0)	117 (45.2)	49 (41.5)
Cigarette smoker	72 (19.1)	53 (20.5)	19 (16.1)
Drug Abuse	68 (18.0)	44 (17.0)	24 (20.3)
Body Site(s) Involved, n (%)			
Head	8 (2.1)	6 (2.3)	2 (1.7)
Chest	20 (5.3)	13 (5.0)	7 (5.9)
Arm	64 (17.0)	38 (14.7)	26 (22.0)
Abdomen	42 (11.1)	40 (15.4)	2 (1.7)
Perineum	139 (36.9)	111 (42.9)	28 (23.7)
Leg	124 (32.9)	67 (25.9)	57 (48.3)
Multiple Sites	19 (5.0)	15 (5.8)	4 (3.4)
Infection Etiology, n (%)			
Boil/Furuncle	65 (17.2)	50 (19.3)	15 (12.7)
Trauma	45 (11.9)	20 (7.7)	25 (21.2)
Chronic Wound	42 (11.1)	23 (8.9)	19 (16.1)
Injection, Drug Use-Related	48 (12.7)	36 (13.9)	12 (10.2)
Perirectal Abscess	17 (4.5)	15 (5.8)	2 (1.7)
Post-Operative	21 (5.6)	18 (6.9)	3 (2.5)
Idiopathic	118 (31.3)	83 (32.0)	35 (29.7)
Other	21 (5.7)	13 (5.5)	7 (5.8)
Polymicrobial Infection, n (%)	275 (72.9)	193 (74.5)	83 (69.5)

Abbreviations: IQR, interquartile range; CRF, chronic renal failure

Table II: Summary of organisms observed in surgical wound cultures.

		Polymicrobial		Beta-Hemolytic Streptococci	
	Total (n=377)	**No (n=102)**	**Yes (n=275)**	**Absent (n=259)**	**Present (n=118)***
Streptococcus spp.	209 (55.4)	43 (42.2)	166 (60.4)	91 (35.1)	NA
GAS	75 (19.9)	32 (31.4)	43 (15.6)	NA	NA
GBS	37 (9.8)	4 (3.9)	33 (12.0)	NA	NA
SD	8 (2.1)	0 (0)	8 (2.9)	NA	NA
Staphylococcus spp.	155 (41.1)	21 (20.6)	134 (48.7)	100 (38.6)	55 (46.6)
MRSA	41 (10.9)	11 (10.8)	30 (10.9)	26 (10.0)	15 (12.7)
MSSA	37 (9.8)	7 (6.9)	30 (10.9)	19 (7.3)	18 (15.3)
Gram Negative Rods	135 (35.8)	4 (3.9)	131 (47.6)	106 (40.9)	29 (24.6)
Mixed Anaerobic Flora	192 (50.9)	0 (0)	192 (69.8)	157 (60.6)	35 (29.7)
Candida spp.	38 (10.1)	4 (3.9)	34 (12.4)	28 (10.8)	10 (8.5)

Abbreviations: GAS, group A streptococcus; GBS, group B streptococcus; SD, *Streptococcus dysgalactiae subspecies*; MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-sensitive *Staphylococcus aureus*
*Some patients had more than one beta-hemolytic streptococcal species

Table III: Clinical outcomes of NSTI patients with culture data overall (n=377), and by presence (n=118) and absence (n=259) of beta-hemolytic streptococci.

		Beta-Hemolytic Streptococci	
	Total (n=377)	**Absent (n=259)**	**Present (n=118)**
Ventilator-Free Days, median [IQR]*	26 [24, 28]	26 [24, 28]	26 [24, 28]
ICU-Free Days, median [IQR]*	24 [20, 25]	24 [20, 25]	24 [20, 25]
Hospital LOS (days), median [IQR]	19 [11, 30]	18 [10, 31]	21 [11, 39]
Number of Debridements, median [IQR]	3 [2, 4]	3 [2, 4]	3 [2, 4]
***Clostridioides difficile* colitis, n (%)**	12 (3.2)	7 (2.7)	5 (4.2)
STSS, n (%)	N/A	N/A	6 (5.1)
Amputation, n (%)^	42/187 (22.5)	16/105 (15.2)	26/82 (31.7)
Mortality, n (%)	55 (14.6)	43 (16.6)	12 (10.2)

*Out of 28 days

^Among patients with extremity involvement

Abbreviations: IQR, interquartile range; ICU, intensive care unit; LOS, length of stay; STSS, streptococcal toxic shock syndrome

Table IV: Unadjusted and adjusted risk ratio estimates for the occurrence of amputation and mortality, comparing patients with and without beta-hemolytic streptococci.

	Unadjusted RR [95% CI]	Adjusted RR* [95% CI]
Amputation^		
Beta-hemolytic streptococci absent	REF	REF
Beta-hemolytic streptococci present	2.08 [1.20-3.61]	1.79 [1.07-3.01]
Mortality		
Beta-hemolytic streptococci absent	REF	REF
Beta-hemolytic streptococci present	0.61 [0.34-1.12]	0.72 [0.40-1.31]

^Among patients with extremity involvement (n=187)

*Adjusted for injection drug use, transfer status, and age by quintile

Table V: Unadjusted and adjusted risk ratio estimates comparing patients with and without clindamycin resistant strains among patients with beta-hemolytic streptococci.

	Unadjusted RR [95% CI]	Adjusted RR* [95% CI]
Amputation^		
Clindamycin sensitive	REF	REF
Clindamycin resistant	1.07 [0.49-2.32]	1.86 [1.10-3.16]
STSS		
Clindamycin sensitive	REF	REF
Clindamycin resistant	1.10 [0.21-5.69]	1.23 [0.25-6.08]
Mortality		
Clindamycin sensitive	REF	REF
Clindamycin resistant	0.83 [0.21-2.90]	1.38 [0.41-4.63]

^Among patients with extremity involvement (n=73)
*Adjusted for injection drug use, transfer status, and age by quintile
Abbreviations: STSS, streptococcal toxic shock syndrome

Figure I: Flowchart outlining patient culture characteristics.