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PCR Offers No Advantage Over Culture for Microbiologic Diagnosis in Cellulitis

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Abstract

Purpose—Most cases of cellulitis are traditionally attributed to β-hemolytic streptococci and *Staphylococcus* species, although in most cases, no organism is identified. Development of PCR using the conserved bacterial 16s-rRNA DNA permits identification of bacteria independent of conventional culture approaches and prior use of antibiotics.

Methods—We used PCR-based techniques to identify cellulitis etiology using aspirate samples from affected skin. Saline was infiltrated and aspirated at the site of greatest erythema or at the cellulitic border. Samples were tested for 16s-rRNA DNA and organism-specific probes used to identify bacteria commonly seen in skin infections.

Results—Aspirates from 32 patients were studied and16s-rRNA DNA was detected in 9/32 of the cases (28.1%). Bacterial species were identified by PCR methods in 6/9 (66.6%). *S. aureus* was identified in 4/6 samples, and 2/6 were methicillin-resistant *S. aureus* (MRSA). Of patients with positive aspirate bacterial cultures (3/9, 33.3%), *S. aureus* was present on culture in 2/3 (66.6%) positive samples, and coagulase-negative *Staphylococcus* (CoNS) was present on culture in 2/3 (66.6%) positive samples. Only in one of the three positive bacterial cultures did the PCR method detect the same organism as was detected by culture. Among patients with positive provider-collected clinical cultures, MRSA was the predominant organism (11/18, 61.1%) and when present, it was found as the sole organism. Where *S. aureus* or *Streptococcus* species were detected by molecular methods, clinical cultures yielded a positive result as well.

Conclusions—PCR-based techniques do not appear to be more sensitive than aspirate cultures for detection of pathogens in cellulitis.

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Keywords

cellulitis; eukaryotic PCR; 16S rRNA; skin and skin structure infection; SSTI

Introduction

Cellulitis is a common inflammatory skin disorder, usually attributed to infection with Group A, β-hemolytic streptococci and *Staphylococcus aureus* [1, 2]. Because of the recent increase in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) skin and soft tissue infections [3, 4], practice guidelines increasingly recommend treating uncomplicated cellulitis [5] with MRSA-effective regimens, leading to greater use of non-beta-lactam, broad spectrum antibiotics [6].

In most cases of cellulitis, an etiologic laboratory diagnosis is not aggressively pursued by physicians; furthermore less than 10% cases eventually become bacteremic [7]. Previous approaches to defining the bacterial etiology of cellulitis used needle aspirates of the leading edge for Gram stain and bacterial culture. The sensitivity of this approach is low, and it largely has been abandoned [1, 8–10]. We hypothesized that this low sensitivity was due to low bacterial loads or non-viable or lysed bacteria in the inflamed area, and that new nucleic acid amplification techniques, such as polymerase chain reaction (PCR) would be able to detect the causative bacteria from aspirates of the leading edge or site of maximal erythema.

Methods

We recruited and enrolled a systematic sample of adult patients whose admitting diagnosis was cellulitis, performed needle aspirates and evaluated them by PCR. Patients were identified within 24 hours of admission. The protocol was approved by the Johns Hopkins Institutional Review Boards (IRB).

Exclusion criteria were an inability to provide informed consent, presence of a coagulopathy, hypotension or presence of systemic inflammatory response syndrome (SIRS). Data collected included demographics, clinical and laboratory information, antimicrobial usage and hospital readmissions. Outcome measures were the predominant species identified by culture and/or PCR. A difference in proportions of samples agreeing on PCR and provider culture versus aspirate culture and provider culture were evaluated using Fisher's exact test.

After informed consent, 3–5 mL of 0.85% sterile non-bacteriostatic saline was infiltrated subcutaneously at the site of greatest erythema or at the cellulitic border using a 24-gauge needle. Frankly suppurative areas were avoided in patients with abscesses or wounds (13/32, 40.6%). Fluid was aspirated and suspended in 0.5 mL of RNA*later*® solution (Qiagen, Valencia, CA) or sterile saline. For negative controls, 0.25–0.5 mL of sterile saline was similarly infiltrated into and aspirated from an unaffected skin area on the forearm and suspended in 0.5 mL of RNA*later*® solution or sterile saline. Samples were refrigerated at 4°C and subsequently frozen at −20°C until DNA extraction and PCR analysis. For comparison of clinical and PCR results, a random subset of nine patient cellulitis samples

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were transported directly to the clinical laboratory within 30 minutes of collection, where they were processed using standard microbiological methods [11, 12].

DNA was extracted from a 200μl aliquot of the aspirate using the DNAeasy DNA purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All reactions were performed in duplicates using the LightCycler 480 System (Roche Applied Science, Indianapolis, IN, USA). Signals were considered positive if the amplification curve was 3 cycles below the negative control (approximately 35 cycles).

In samples where 16S-rRNA DNA was detected, further analysis was performed for individual bacterial species identification, using primers and probes that have been previously described and validated in our laboratory [13]. The limit of detection varies according to the PCR assay and ranges from 10^1 – 10^2 CFU/mL. These tested for the presence of: streptococci *(S. pyogenes and S. pneumoniae*), Group B streptococci (GBS), *S. aureus, Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis, Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterococcus faecalis,* and *Enterococcus faecium*. All bacterial primers and probes were synthesized by TIB MOLBIOL (Adelphia, NJ).

Samples that were positive for 16S-rRNA DNA but were negative by these select primers for bacterial species identification were further analyzed using 16S-rRNA sequencing. PCR reactions were carried out using SYBR green on the LightCycler system, and PCR products were separated by gel electrophoresis. DNA bands were excised from the gel and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and then sequenced by the Johns Hopkins Sequencing Center. The sequences were compared to known sequence libraries using the Blast program http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Routine clinical bacterial cultures (aerobic culture only) were performed on provider-collected swabs for identification of bacterial organisms from associated abscesses or wounds. However, not all patients had clinical cultures available. Concurrent bacterial culture was performed on aspirates collected from nine patients enrolled, by directly inoculating 100μl of aspirate onto sheep blood agar (SBA) and chocolate agar (CHA) plates. Agar plates were incubated at 35°C at 5–10% CO_2 for 24 h; cultures negative for growth after 24 h were reincubated for an additional 24 h before finalized as negative for growth. Identification of organisms was performed using routine clinical microbiology test procedures, including Gram stain, catalase, coagulase, oxidase, indole, and the API20E and API20NE test strips (bioMérieux, Durham, NC). Culture and organism identification were performed using standard clinical laboratory procedures and methods, as established in the clinical microbiology laboratory and guidelines by the Clinical and Laboratories Standards Institute (CLSI).

Results

32 patients were included in this study and baseline patient characteristics and characterization of their disease is described in Table 1. More than half of patients had any history of prior cellulitis (59.4%), including MRSA skin infections (31.3%). Thirty one

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(97%) patients received antibiotics before sample collection and the median time from first antibiotic administration to sample collection was 14 hours (IQR 12.6–23.6). Of patients with abscesses, provider-collected cultures were available for 100% (13/13). Incision and drainage of abscesses was performed in 9/32 (28.1%) of cases and of these, 8/9 (88.8%) were MRSA and 1/9 (11.1%) was viridians streptococci. Of all subjects studied, 27/32 (84.3%) had blood cultures collected and two (7.4%) had a diagnosis of concurrent bacteremia (one patient with methicillin-sensitive *S. aureus* (MSSA) and another patient with *S. pyogenes* (Group A).

We detected 16s-rRNA DNA in 9/32 (28.1%) of patient samples, and bacterial species were identified by PCR methods in 6/9 (66.6%). For the other 3 positive samples, definitive organism identification was not achieved by either species-specific PCR or sequencing, as the signal detected by 16s-rRNA DNA PCR was too low to obtain a PCR product appropriate for sequencing. Of the two patients with bacteremia in association with cellulitis, aspirate PCR was negative. PCR controls (collected from the forearm) were negative with the exception of a single sample, which may represent contamination at the time of aspirate collection. Of note, this control was probe-negative for the species tested in our panel of organisms.

S. aureus was the predominant organism identified by PCR probe method. MRSA alone was present in 2/9 (22.2%) samples, while MSSA was found in two samples, and once in combination with *Alcaligenes faecalis* (identified by sequencing). One sample was positive for *E. coli* and another single sample positive for *Streptococcus* spp. [the probe does not distinguish between *S. pneumoniae* versus *S. pyogenes*].

Of the nine aspirate cultures collected, 3/9 (33.3%) were positive for bacterial organisms. In two of these positive aspirate cultures, *S. aureus* and CoNS were identified, respectively; in both cases, *S. aureus* was detected by the PCR method. In the third positive aspirate sample culture, both CoNS and *S. aureus* were identified; however only *S. aureus* was detected by the PCR method (the panel of PCR assays cannot detect (CoNS).

The study results were compared to the corresponding clinical provider-obtained swab cultures (21 available patient samples, Table 2). The provider-collected swab cultures commonly reflect a more diverse bacterial flora, but 11/18 (61.1%) of patient samples with any positive swab cultures had negative results by the 16S-rRNA DNA PCR method. In cases of detection of *S. aureus* or *Streptococcus* spp. by molecular methods, all the clinical provider-collected swab cultures yielded a positive result as well (Table 2). For 21 provider collected swab samples, four corresponding aspirate cultures were available. Of those 4 samples, 2 had no growth by the aspirate culture but were positive for MRSA and *Corynebacterium s*pp., respectively, by the provider-collected sample. Two samples were positive for MRSA and MSSA, respectively, by both methods (Table 2). In sum, in 5 of 21 (23.8%) provider cultures, probe identification matched culture result, while 2 of 9 (22.2%) aspirate cultures matched provider cultures, p=1.0, Fisher's exact test.

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Discussion

Our objective was to determine the bacterial etiologies of cellulitis by molecular methods compared to conventional culture methods and aspirate cultures. To our knowledge, this is the first study to use 16s-rRNA DNA PCR to study the bacterial etiology of cellulitis. Surprisingly, PCR did not substantially improve the frequency of bacterial detection, and our results are essentially no different compared to older studies using traditional aspirate and biopsy cultures [2, 10, 14]. Since PCR can detect DNA from both viable and non-viable/lysed organisms, it would be unlikely to be affected by recent antibiotic treatment. Therefore, our results further support the concept of low organism density in cellulitis as previously described [8, 10, 15].

These negative results are of value in consideration of the pathogenesis and management of cellulitis. Based on our study results together with previously published studies, we suggest that cellulitis-induced inflammation is likely due to effects of bacterial toxins or other factors that diffuse into affected tissue via lymphatics or interstitial fluid, and that bacterial burden at the affected site is less influential. The inflammatory cascade, once triggered, rather than the bacterial bio-burden, may largely define the pathogenesis of cellulitis. If so, we would propose that current treatment practices may need to be revised, in particular those that are based on vancomycin or broad-spectrum agents, where swab cultures are unobtainable or noncontributory.

There are some limitations to our study. As a limited set of species-specific probes was used, sequencing was necessary in some cases and ultimately restricted by remaining sample quantity. Additionally, among PCR-probe positive samples, mixed bacterial populations may have gone undetected, as these samples were not further sequenced to identify other, more unusual bacterial species not included in the probe set. Another potential limitation of the study was the absence of routine provider-collected and aspirate anaerobic cultures, and it is possible that this led to lack of identification of anaerobic bacteria from the skin. Furthermore, since nearly all patients in our study received antibiotics prior to collection of the wound specimens/samples, it was not possible to determine whether or to what extent administration of antibiotics influenced our rate of organism recovery. However, this limitation is nearly unavoidable, as the determination of cellulitis remains a clinically guided diagnosis, and antibiotics are administered in the ED rapidly following the current standard of care. Lastly, misclassification of conditions mimicking cellulitis (e.g., venous stasis dermatitis, vascular disease with dependent rubor, etc) may have led to study case dilution and diminished the sensitivity of our approach.

In conclusion, our results emphasize the need for an improved understanding of the pathogenesis of cellulitis and its bacterial etiologies. Additional studies of the inflammatory mediators of cellulitis, including bacterial species-specific toxins, may help guide targeted therapy, limit the use of broad-spectrum antibiotics and could lead to new approaches in the treatment of cellulitis.

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Table 1

Patient Characteristics

Age, years (median, IQR)	54.0 (45.0–61.0) N (%)
Female	12 (37.5)
Race	
Caucasian	30 (93.8)
African American	1 (3.1)
Other	1 (3.1)
Diabetes	14 (45.2)
Peripheral vascular disease	3 (9.7)
Coronary artery disease	8 (25.0)
Venous disease [a]	7 (21.9)
Location of cellulitis	
Hand	1 (3.1)
Arm	2 (6.3)
Leg	16 (50.0)
Foot	4 (12.5)
Other location	9 (28.0)
Abscess present (1 or more)	13 (40.6)
Incision and drainage performed	9 (28.1)
History of prior cellulitis	19 (59.4)
Prior MRSA skin infections	10 (31.3)
Bacteremia [b]	2 (7.4)
History of recent or remote trauma to affected site	13 (40.6)
Hours from ED[c] antibiotics to study sampling, hours (median, IQR)	14 (12.6–23.6)
Hospital readmission for any SSTI within7 months after discharge	9 (28.1)

[a]Per exam or medical record.

[b]27/32 patients had blood cultures performed

[c]Emergency Department.

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Table 2

Swab clinical cultures versus all aspirate PCR and aspirate cultures from cellulitic skin.

Patient	Provider-collected clinical culture	Control PCR	Sample PCR	Probe identification	Aspirate culture
1	MRSA	[a]	–	N/A[b]	ND[c]
2	MRSA	–	–	N/A	ND
3	ND	–	–	N/A	ND
4	MRSA	–	–	N/A	ND
5	MRSA	–	–	N/A	ND
6	ND	–	–	N/A	ND
7	MRSA	–	+	MRSA	ND
8	*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus* Group B	–	–	N/A	ND
9	MRSA	–	–	N/A	ND
10	ND	–	–	N/A	ND
11	*Pseudomonas aeruginosa*	–	–	N/A	ND
12	ND	–	–	N/A	ND
13	MRSA	–	–	N/A	ND
14	MRSA	–	+	MRSA	ND
15	MSSA, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	+[d]	+	*E. coli*	ND
16	No growth	–	+	None[e]	ND
17	Streptococcus Group G	–	–	N/A	ND
18	MRSA	–	+	None	ND
19	viridans streptococci	–	+	*Streptococcus*	ND
20	MSSA	–	+	MSSA	ND
21	ND	–	–	N/A	ND
22	ND	–	–	N/A	ND
23	MSSA	–	+	MSSA, *Alcaligenes faecalis*[f]	*Staphylococcus aureus*[g], Coagulase-negative staphylococcus
24	MRSA	–	+	None	No growth
25	ND	–	–	N/A	No growth
26	ND	–	–	N/A	No growth
27	ND	–	–	N/A	No growth

Patient	Provider-collected clinical culture	Control PCR	Sample PCR	Probe identification	Aspirate culture
28	ND	–	–	N/A	Coagulase-negative staphylococcus
29	MRSA	–	–	N/A	No growth
30	*Corynebacterium* species	–	–	N/A	No growth
31	MRSA	–	–	N/A	*Staphylococcus aureus*
32	ND	–	–	N/A	ND

[a] No control was available for Patient 1.

[b] N/A, not applicable.

[c] ND, Not done.

[d] Positive control was probe-negative for *E. coli* and other species tested in the probe panel of organisms.

[e] Probe was negative and sequencing was unsuccessful, therefore final identification was not possible.

[f] Identified by sequencing.

[g] Antibiotic susceptibilities were not performed on aspirate culture isolates.

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