Diabetic Foot Infections: Microbiology Made Modern?

Array of hope

Many aspects of managing foot infections in patients with diabetes have improved dramatically and are summarized in recently published guidelines (1–3). Yet, the basic methods of determining the causative microorganisms from these infections by culturing wound specimens have remained largely unchanged for over a century. Obtaining a proper wound culture specimen allows the clinician to define the pathogens involved and their antibiotic susceptibility. Unfortunately, results of cultures are generally not available for at least 2–3 days. Thus, most antibiotic therapy for infections is selected empirically (4). By the time culture results arrive, they are usually too late to influence the initial antibiotic choice. Many clinicians, therefore, feel compelled to select an antibiotic regimen that will cover most of the likely bacteria for all but the mildest infections, leading to an unnecessarily broad spectrum of therapy. This overprescribing increases the likelihood of adverse drug effects, drives antibiotic resistance, and increases the cost of treatment. To the contrary, some clinicians conclude that it is not worthwhile to obtain cultures, believing that the belated results are unhelpful. This approach, however, makes it difficult to properly select an alternative regimen if the patient is failing to respond to the initial agent(s).

The increasing incidence of antibiotic-resistant pathogens as causes of diabetic foot infections makes selecting empiric antibiotic therapy more difficult. Those who treat these patients are well aware of the growing problem of methicillin-resistant *Staphylococcus aureus* (MRSA), which is now frequently acquired in the community and in various types of health care facilities (5,6). In addition, in many clinics (especially in developing countries), highly resistant strains of gram-negative bacilli, especially *Pseudomonas aeruginosa*, are an increasing problem (7–9). Similarly, *Enterococcus* species are often isolated from diabetic foot wounds, especially in patients recently treated with antibiotics. It is often unclear whether some of these latter, less-virulent isolates, unlike *S. aureus*, represent true pathogens that require targeted therapy (10). An additional dilemma facing practitioners is that current guidelines recommend only treating diabetic foot wounds that are clinically infected, i.e., those with purulent secretions or with signs or symptoms of inflammation. Unfortunately, wounds in patients with peripheral neuropathy or peripheral arterial disease may fail to show classic symptoms of inflammation, which makes diagnosis difficult (11). Thus, it may be difficult to know which wounds require antibiotic therapy.

In this issue of *Diabetes Care*, Sotto et al. (12) show us a glimpse of what we may expect in the future for clinical microbiology. Among diabetic patients hospitalized with a foot ulcer, they selected those who had not had any recent antibiotic therapy and who had a wound culture specimen that grew only *S. aureus*. Almost one-half of these isolates were MRSA. They classified the wounds according to the Infectious Diseases Society of America/International Working Group on the Diabetic Foot (1,2) as those that were clinically uninfected (grade 1) and those that were infected (grade $\geq$2). This wound classification system has recently been validated as predicting clinical outcomes of diabetic foot infections (13). The aim of the investigators was to differentiate colonized from infected wounds using a miniaturized oligonucleotide array, a genotyping method that can detect the presence of genes encoding for various virulence factors and antibiotic resistance in $<$1 day (14). Clinically uninfected patients were not treated with antibiotic therapy, and only 8 of 22 (36%) patients healed their ulcer. They found that genes for both virulence and resistance factors were present significantly more often in clinically infected wounds. Virulence factors were present in *S. aureus* isolates of only two (9%) grade 1 patients. To the contrary, virulence factors were found in 49 of 50 (98%) infected patients. Among patients with grade 1 lesions, 13 (59%) had persistent colonization with *S. aureus* on follow-up cultures. Virulence factors were not present in either of the two whose wounds healed, whereas virulence factors were initially present or acquired at follow-up in all of those who failed to heal. These data highly suggest that virulence factors are markers for infected wounds. If so, they would offer an objective means of making this critical determination, particularly in patients with a neuroischemic limb. Furthermore, the presence of virulence factors appears to predict an adverse clinical outcome for which we have few other markers (15).

This study provides a number of additional useful observations. First, whereas some grade 1 ulcers healed without antibiotic therapy, the majority did not. This outcome could be related to inadequacies of other aspects of wound care in these patients with a foot ulcer, but it could also represent a failure to properly identify clinically uninfected wounds. Second, *S. aureus* was a remarkably persistent colonizer, being recovered on follow-up culture in almost 60% of patients with a grade 1 ulcer. Third, only a few *S. aureus* isolates from ulcers that presented as a first episode were MRSA, compared with all of those from recurrent wounds. Patients with recurrent ulcers were likely to have been previously hospitalized and treated with antibiotics, which are known risk factors for MRSA in many, but not all, studies (16). Fourth, ulcers that remained colonized with MRSA were unlikely to heal, affirming a worse outcome with MRSA than with other pathogens, as previously noted (17). Specifically, Tentolouris et al. (16) found that in 84 patients with an infected or uninfected diabetic foot ulcer, *S. aureus* was the most common gram-positive wound isolate, that almost 50% of isolates were MRSA, and that the prevalence of MRSA was significantly higher in the infected ulcers. Fifth, there was a trend toward antibiotic resistance genes (especially for aminoglycosides) to be associated with greater clinical severity of infection. Sixth, virulence genes were sur-

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prisingly common in the *S. aureus* isolates; those for enterotoxins were found in nearly one-half of the isolates and those for leukocidins in about two-thirds. Finally, the antibiotic resistance results on the array were in complete agreement with those of standard sensitivity testing, attesting to the accuracy of the procedure.

This study confirmed previously reported results of arrays and PCR tests (18,19). So, what further microbiological advances would help clinicians optimize antimicrobial therapy for diabetic foot infections? With PCR methods, it is possible to detect most species of pathogens in a wound in a matter of hours rather than days. PCR has already shown promise in various clinical situations, including identifying antibiotic-resistant gram-negative organisms (20), bacteria that elaborate neurotoxins (21) or other virulence factors (22), and slow-growing organisms like mycobacteria (23). Additionally, previous antibiotic therapy would be much less likely to cause false-negative results than with a standard culture. PCR is also able to detect much smaller concentrations of microorganisms than standard cultures. It is certainly possible that PCR or similar techniques could provide not only the name of the pathogens but also their susceptibility patterns, all within a time frame that would allow replacing most empirical therapies with evidence-based therapy. Taken together, these faster, "smarter," and more sensitive techniques could revolutionize how we target antimicrobials against increasingly diverse and resistant pathogens. However, better microbiology detection methods will be of no value unless clinicians send laboratories optimally obtained specimens, i.e., tissue samples collected after wound cleansing and debridement. Applying what we already know about sending a good specimen to get a good result should improve our antibiotic prescribing now while we wait for the exciting innovations in clinical microbiology that appear to be coming in the near future.

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References

1. Lipsky BA: A report from the international consensus on diagnosing and treating the infected diabetic foot. *Diabet Metab Res Rev* 20 (Suppl. 1):S68–S77, 2004
2. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS, Infectious Diseases Society of America: Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 39:885–910, 2004
3. Societe de Pathologie Infectieuse de Langue Francaise: [Management of diabetic foot infections: short text[. *Med Mal Infect* 37:1–25, 2007 [article in French]
4. Lipsky BA: Empirical therapy for diabetic foot infections: are there clinical clues to guide antibiotic selection? *Clin Microbiol Infect* 13:351–353, 2007
5. Eady EA, Cove JH: Staphylococcal resistance revisited: community-acquired methicillin resistant Staphylococcus aureus: an emerging problem for the management of skin and soft tissue infections. *Curr Opin Infect Dis* 16:103–124, 2003
6. Dang CN, Prasad YD, Boulton AJ, Jude EB: Methicillin-resistant Staphylococcus aureus in the diabetic foot clinic: a worsening problem. *Diabet Med* 20:159–161, 2003
7. Tascini C, Gemignani G, Palumbo F, Leonildi A, Tedeschi A, Lambelet P, Lucarini A, Piaggesi A, Menichetti F: Clinical and microbiological efficacy of colistin therapy alone or in combination as treatment for multidrug resistant *Pseudomonas aeruginosa* diabetic foot infections with or without osteomyelitis. *J Chemother* 18: 648–651, 2006
8. Viswanathan V: The diabetic foot: perspectives from Chennai, South India. *Int J Low Extrem Wounds* 6:34–36, 2007
9. Shankar EM, Mohan V, Premalatha G, Srinivasan RS, Usha AR: Bacterial etiology of diabetic foot infections in South India. *Eur J Intern Med* 16:567–570, 2005
10. Lipsky BA, Armstrong DG, Citron DM, Tice AD, Morgenstern DE, Abramson MA: Ertapenem versus piperacillin/tazobactam for diabetic foot infections (SIDESTEP): prospective, randomised, controlled, double-blinded, multicentre trial. *Lancet* 366:1695–1703, 2005
11. Edmonds M: Infection in the neuroischemic foot. *Int J Low Extrem Wounds* 4:145–153, 2005
12. Sotto A, Richard J-L, Jourdan N, Combescure C, Bouziges N, Lavigne J-P, Nîmes University Hospital Working Group on the Diabetic Foot (GP30): Miniaturized oligonucleotide arrays: a new tool for discriminating colonization from infection due to *Staphylococcus aureus* in diabetic foot ulcers. *Diabetes Care* 30:XXXX–XXXX, 2007
13. Lavery LA, Armstrong DG, Murdoch DP, Peters EJ, Lipsky BA: Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. *Clin Infect Dis* 44:562–565, 2007
14. Heller MJ: DNA microarray technology: devices, systems, and applications. *Annu Rev Biomed Eng* 4:129–153, 2002
15. Lipsky BA, Sheehan P, Armstrong DG, Tice AD, Polis AB, Abramson MA: Clinical predictors of treatment failure for diabetic foot infections: data from a prospective trial. *Int Wound J* 4:30–38, 2007
16. Tentolouris N, Petrikkos G, Vallianou N, Zachos C, Daikos GL, Tsapogas P, Markou G, Katsilambros N: Prevalence of methicillin-resistant *Staphylococcus aureus* in infected and uninfected diabetic foot ulcers. *Clin Microbiol Infect* 12:186–189, 2006
17. Wagner A, Reike H, Angelkort B: [Highly resistant pathogens in patients with diabetic foot syndrome with special reference to methicillin-resistant *Staphylococcus aureus* infections.] *Dtsch Med Wochenschr* 126:1353–1356, 2001 [article in German]
18. Monecke S, Slickers P, Hotzel H, Richter-Huhn G, Pohle M, Weber S, Witte W, Ehricht R: Microarray-based characterisation of a Panton-Valentine leukocidin-positive community-acquired strain of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 12:718–728, 2006
19. Monecke S, Ehricht R: Rapid genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates using miniaturised oligonucleotide arrays. *Clin Microbiol Infect* 11:825–833, 2005
20. Horii T, Morita M, Muramatsu H, Monji A, Miyagishima D, Kanno T, Maekawa M: Antibiotic resistance in *aeromonas hydrophila* and *vibrio alginolyticus* isolated from a wound infection: a case report. *J Trauma* 58:196–200, 2005
21. Akbulut D, Grant KA, McLauchlin J: Improvement in laboratory diagnosis of wound botulism and tetanus among injecting illicit-drug users by use of real-time PCR assays for neurotoxin gene fragments. *J Clin Microbiol* 43:4342–4348, 2005
22. Rosche TM, Yano Y, Oliver JD: A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. *Microbiol Immunol* 49: 381–389, 2005
23. Phillips R, Horsfield C, Kuijper S, Lartey A, Tetteh I, Etuaful S, Nyamekye B, Awuah P, Nyarko KM, Osei-Sarpong F, Lucas S, Kolk AH, Wansbrough-Jones M: Sensitivity of PCR targeting the IS2404 insertion sequence of *Mycobacterium ulcerans* in an assay using punch biopsy specimens for diagnosis of Buruli ulcer. *J Clin Microbiol* 43:3650–3656, 2005

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