CLINICAL MANAGEMENT extra

Incorporating Cutaneous and Wound Bacterial Bioburden Biomarkers into Clinical Research: A Review of Best Practices

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PURPOSE:

To provide information about initiating interdisciplinary research related to microbiomes and their role in human immunity, disease, and metabolic processes.

TARGET AUDIENCE:

This continuing education activity is intended for physicians and nurses with an interest in skin and wound care.

OBJECTIVES:

After participating in this educational activity, the participant should be better able to:

1. Describe techniques to identify and characterize bacterial bioburden.

2. Identify optimal collection, transport, and storage of samples.

ABSTRACT

OBJECTIVE: The purpose of this review is to provide a roadmap for clinical scientists interested in integrating bacterial bioburden (BB) biomarkers into the next generation of cutaneous or wound disease research studies.

DISCUSSION: Complex relationships exist between humans and their microbiome. Until now, clinical scientists have been limited in fully characterizing relationships between humans and their microbiome. Recent technological innovations, such as next-generation DNA sequencing, also known as deep sequencing or pyrosequencing, have enhanced clinicians' capacity to identify, characterize, and elucidate the role of BB (ie, bacterial load, diversity, pathogenicity) in human immunity, disease, and metabolic processes. The understanding of common terminology, intervening variables that influence BB, limitations of next-generation DNA sequencing, and specimen selection, collection, transport, and storage practices are needed to support interdisciplinary communication, research design, and integrity of the specimen.

CONCLUSION: This review serves as a primer for building foundational knowledge in microbiome research, which will aid clinical scientists with initiating interdisciplinary communication necessary for scientific team building.

KEYWORDS: next-generation sequencing, 16s rDNA, microbiome, wounds, cutaneous, bacterial bioburden

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INTRODUCTION

Complex relationships exist between humans and the bacterial, archaeal, viral, and fungal communities (microbiome) that inhabit a common location, such as the skin or gut. Until now, clinical scientists have been limited in fully characterizing relationships between humans and their microbiomes. Recent technological innovations, such as next-generation DNA sequencing (NGS), also known as deep sequencing or pyrosequencing, have enhanced the clinician's capacity to identify, characterize, and elucidate the commensal or pathological role that bacteria play in human immunity, disease, and metabolic processes via the amplification of genetic material.[1] Unlike traditional cultivation-based laboratory assays, NGS methods provide clinical scientists an unprecedented ability to investigate the abundance of bacteria present in a given body region, commonly known as the bacterial bioburden (BB), with accuracy and precision.[2] Furthermore, NGS technology provides the tools to address a recent challenge from the American Geriatric Society to identify the implications of the microbiome in health and age-related diseases, including cutaneous and wound-related diseases.[3] In preparing to conduct BB research, building an interdisciplinary research team (with bioinformaticians and medical microbiologists) is necessary to support successful projects. The purpose of the review is to provide a roadmap for clinical scientists interested in integrating BB biomarkers (bacterial load [BL], diversity, pathogenicity) into cutaneous or wound disease research studies and to build the foundation for interdisciplinary communication by expanding upon common language and techniques used to conduct BB research. In the Table, the authors provide a glossary of common terminology and encourage those who seek more technical and exhaustive reviews of the area to Lavigne et al,[4] Huttenhower et al,[5] Goodrich et al,[6] and Hamady and Knight.[7]

LANDMARK 1: ESTABLISHING COMMON TERMINOLOGY

Next-generation DNA sequencing. The characterization of bacterial communities utilizes the 16S rRNA gene. The16S rRNA gene encodes RNA that is part of the bacterial 30S small subunit ribosome and contains 9 hypervariable regions that can be sequenced to determine specific bacterial species (operational taxonomic unit).[6,7] See Woese[8] for an exhaustive review of 16S rDNA. The 16S rDNA is isolated via common genomic DNA extraction methods, many of which are available via straightforward manufacturer kits, and multiple copies of the DNA are made (amplified) in vitro via the polymerase chain reaction (PCR).

For NGS, short single-stranded segments of DNA (primers) flank the desired 16S rDNA variable region, amplifying the targeted genomic sequence required for analysis, if present.[6]

Bacterial bioburden. In the literature, BB is operationalized into 3 biomarkers: (1) the total quantity of bacteria present, or BL; (2) the number of distinct bacterial taxa present in a specimen, known as bacterial diversity; and (3) the existence of pathogenic organisms in the skin/wound environment.[9] With 16S rDNA data, BL is operationalized as the total number of 16S rDNA copies (amplicons). Bacterial diversity is the number of taxa or operational taxonomic units and can be presented as a portion (relative abundance) of the total number of unique amplicons and is often presented as a stacked column graph. Bacterial diversity is further differentiated as the number of taxa in 1 sample (alpha diversity) or the similarities or differences of the bacterial taxa between samples (beta diversity).[10] The ability of individual or groups of bacteria, viruses, or other microorganisms to cause a disease state (pathogenicity) can be assessed by analyzing associations that exist among the bacterial community membership, structure, and clinical outcome of interest, such as wound infection symptoms.[11] By analyzing associations between bacterial membership, structure, and clinical outcomes of interest, scientists have the ability to form hypotheses

Table.
GLOSSARY OF TERMS

Taxa	Any unit used for biological hierarchical classification (eg, kingdom, species), taxonomy[56]
16s rDNA	The sequence of the bacterial genome that codes for the 16s rRNA ribosomal subunit. This region of code evolves very slowly and is thus used to establish bacterial phylogeny[8]
Operational taxonomic unit	Operational taxonomic unit is a proxy used for assigning the particular level of taxa unique to the amplified genetic code[9]
Primer	Short, single-stranded pieces of DNA used to initiate the PCR reaction. Bind to a target DNA[6]
Barcode	A noncoding, single-stranded DNA sequence attached to the NGS primer, used to differentiate one sample from another during and after sequencing[56]
Relative abundance	The numerical value (fraction or percentage) that represents the amount of 1 type of organism in the context of the total population[9]
Amplicons	Product of nucleic acid (DNA/RNA) amplification[56]
Polymerase chain reaction	www.jove.com/science-education/5056/pcr-the-polymerase-chain-reaction
Alpha diversity	Variation of microbial population within a given subset/subject/sample location[57]
Beta diversity	Variation of microbial population between subsets/subjects/sample locations[57]
Colony-forming unit	An approximate measure of concentration based on the number of individual colonies that grow or can be cultured on solid media. This can be estimated by other means, but the resulting value is still correlated back to solid media growth[58]
Reads	Term used to refer to individual sequenced strands of DNA[56]

about pathogenicity. A combination of the 3 dimensions provides a comprehensive assessment that supports characterizing the dynamics of BB in the context of cutaneous and wound diseases.[12]

LANDMARK 2: WHERE DO CLINICIANS WANT TO GO AND HOW DOES NEXT-GENERATION SEQUENCING CHANGE THE ROAD AHEAD?

Microbes have been found in a variety of diverse and improbable habitats, from every inch of the human body[2] to deep sea thermal vents,[13] and evidence pointing to bacterial-like life on Mars continues to build.[14] A clinician's ability to characterize BB in distinct environments is limited by cultivation methods, which are capable of profiling fewer than 10% of microbes present in any sample.[2] An understanding of each bacteria species' specific growth requirements is necessary to support bacterial proliferation for cultivation-based (colony-forming units [CFUs]) BB analysis. Although some organisms, such as *Staphylococcus aureus* or *Escherichia coli*, can proliferate under a wide variety of conditions, more fastidious species, such as strictly anaerobic species, are no longer viable if special precautions (eg, environmental) are not taken to preserve and nurture growth. As a result of these precautions, CFU-based BB results may be biased.[15,16] Environmental requirements can be vastly different among species; however, all bacteria possess genetic material that codes for the bacterial specific ribosomal subunit (16s rDNA), which allows for the characterization of BB regardless of viability at the time of sample collection.[13]

The optimal BB assay has high specificity, sensitivity, and reproducibility and low levels of bias.[7] The most common BB assays reported in the literature are in the form of culture-dependent CFU or the 16s rDNA-based PCR methods. As viability of organisms is not a concern for PCR-based methods, this is currently the in vogue assay providing the lowest bias and highest sensitivity, specificity, and reproducible results compared with other methods.[5] To date, sequencing can be broken down into 2 broad categories: Sanger sequencing and NGS. The Sanger sequencing method begins with the pure culture (laboratory method that removes biological contaminants) process to isolate 1 or multiple single species from 1 mixed culture sample.[17] Then, the bacterial species is amplified via PCR. For a more in-depth exposition of PCR via the Sanger sequencing method, see Sanger et al.[17]

Although NGS can also use pure culture,[18,19] similar to Sanger sequencing, NGS does not have to use a cultured specimen.[6] New-generation sequencing integrates arbitrary nucleotide sequences (barcodes) for each sample. This error-correcting barcode reduces the possibility that the particular sequenced genetic material will be assigned to the wrong sample.[6] Through the integration of unique barcodes, the NGS sequencing process allows for the simultaneous sequencing and differentiation of sequences from multiple samples (up to 384 or more with the most recent sequencing kits). The barcodes assigned to a particular sequenced sample are separated downstream with bioinformatic tools into their respective samples (such as Quantitative Insights Into Microbial Ecology).[6] This allows for easy comparison of BL and alpha and beta diversity in study populations between the NGS and Sanger methods.

LANDMARK 3: NAVIGATING THE INTERVENING VARIABLES OF BACTERIAL BIOBURDEN BIOMARKERS

No 2 individuals' microbiomes are the same. Variability in BB is greatly influenced by the alpha and beta diversity of a microbial

community structure in a selected environment (biogeography) over time, environment, and individual host factors, each of which needs to be considered and measured or controlled for accurate interpretation of the BB. Three dominant skin biogeographical bacterial clusters have been associated with site-specific locations that correlate with sebaceous (eg, face, upper body), moist (eg, flexural surfaces), and dry regions (eg, buttocks, forearms).[12] Each region has a few dominant taxa; however, the majority of organisms are from rare species. For example, moist regions are dominated by phylotypes *β-proteobacteria*, *Staphylococcus*, and *Corynebacterium*, and the remaining relative abundance is dispersed among more than 9 phylotypes.[12] Although dry, moist, and sebaceous clusters have been associated with particular regions, bacterial diversity within similar regions has greater interpersonal than intrapersonal variation.[20] During data collection, to explore questions of beta diversity, specimens should be collected from analogous biogeographical regions; this supports within-subject comparison/control. For the purpose of data analysis, scientists should compare BB outcomes to the known site-specific (eg, sebaceous, moist) bacterial clusters established by SanMiguel et al,[12] other literature specific to their population (eg, diabetic ulcers), or data previously submitted to the National Center for biotechnology information (www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi) as part of the Human Microbiome Project (www.hmpdacc.org).

When conducting BB research, controlling environmental variables that influence bacterial community structure is challenging. Characterizing the host's environment and living conditions will aid in better describing interpersonal variations in BB data. Host BB can be directly influenced by the translocation of bacteria from the environment in which the host lives. For example, the forearm has the greatest temporal variability in microbiome community structure, which has been attributed to constant interaction of environmental objects that introduce bacteria, or other microorganisms, to the forearm.[12] In particular to wound BB, repeated dressing changes or exposure to feces increases the risk of bacterial contamination.[21,22] Furthermore, it has been shown that the host microbiome can be directly influenced by individuals that live in the same space. An animal study of 2 groups of mice demonstrated that cohousing influenced BB phenotype. Mice genetically modified (knockout mice) to exhibit decreased microbial resistance were housed with unmodified control mice. The control mice expressed the same BB phenotype as the knockout mice.[23] Similar findings have been discovered between household members and their family dog.[24] The collection of demographic information, such as degree of close social contact with people or pets, living environment (eg, rural, urban), and personal hygiene, can mitigate the challenges of controlling environmental variables.

In addition to controlling for environmental variables, the influence of individual host factors on BB must also be considered. Studies indicate that there is greater interpersonal microbiome variability than exists between groups of males and females.[12] Factors such as age, taking certain medications (ie, immunomodulators, antibiotics, antiseptics),[25–27] poor lower-extremity circulation (ie, edema, tissue perfusion),[21] and other comorbidities (ie, diabetes, depression, immunodeficiency conditions)[20,28–32] may confound the interpretation of BB data. Together, these factors create a complex web of variables that are difficult to isolate with data that are available, paving the way for additional studies that will deepen the pool of information. With regard to antimicrobial agents, no standard exists for exposure limits or duration prior to sampling. It is the responsibility of the investigator to tailor exclusion criteria to the research question at hand.[9,33] More research is required to elucidate standard exclusion criteria for BB research study enrollment. For a more exhaustive review of the skin microbiome, the authors suggest the previously mentioned Human Microbiome Project and also the published work of SanMiguel and Grice.[12] As many variables influence BB, bacterial specimen integrity and the subsequent laboratory analysis are essential for conducting a reliable and valid BB study. The following sections will highlight the best and most common practices for conducting BB research.

LANDMARK 4: BACTERIA BIOSPECIMEN SAMPLE PREPARATION AND COLLECTION

Bacterial specimens should be handled with universal precautions when used in research and diagnostic applications. For cutaneous and wound (such as fissures, erosions, lesions, ulcers) disease processes, specimens must be handled with care as many infectious agents, both local and systemic, are shed through open wounds.[34] Before collecting the bacterial sample, appropriate steps must be performed to ensure adequate sampling and preservation of sample integrity while in storage. Supplies required to collect a bacterial specimen include specimen collection swabs, collection buffer for swab specimens, single-use punch biopsy or scalpel blade for tissue biopsy specimens, collection tubes, and temperature-controlled transport medium to preserve the sample until it is placed in long-term storage (-80° C refrigeration).[35] The swab material may consist of foam, cotton, alginate, or rayon.[36] No comparative effectiveness research studies were identified that evaluated the influence of the type of swab used for bacterial specimen collection via CFU or PCR methods. However, a common practice proposed in the literature to increase bacterial specimen collection yield is to moisten the swab using a collection buffer if the sample site is dry.[34] Collection buffers identified in literature are traditionally normal saline with 0.1% Tween solution[37] and may contain an

enzymatic lysis buffer mixture of ethylenediaminetetraacetic acid, Triton X-100, or Tris.[38] Areas that are considered moist may be sampled following irrigation of the wound with normal saline.[9] Upon collection of the sample, an appropriate transport medium should be selected.

To obtain an exhaustive review regarding the influence of swab characteristics and transport medium on the specimen, see DeBurger and Mortensen.[36] For 16s rDNA sequencing purposes, alternative transport options for swab specimens protected in collection tubes include using wet ice, (0° C) dry ice (-79° C), or liquid nitrogen (-196° C) for both swab and tissue specimens.[6,37] Although wet ice is appropriate for the transportation of samples to be cultured or sequenced, dry ice and liquid nitrogen may be detrimental to culture samples if not properly prepared.[39] Samples intended for 16s rDNA sequencing must be kept below room temperature to preserve the integrity of the genetic material.[35] Regardless of the approach selected for transport of the specimens, the conditions and approach must be kept consistent across all samples.[6] The aforementioned details regarding specimen collection and transport should be included in method sections of research reports to assist readers in interpreting the results.[40,41]

Cutaneous or wound bacterial specimen types commonly reported in the literature are collected from swabs (eg, superficial collection) and tissue (eg, deep collection) samples.[9,42] The 3 swab techniques for bacterial specimen collection are wound exudate, Z-stroke, and Levine techniques.[43] The wound exudate technique involves using a culture swab and gentle pressure to collect the wound exudate located on the surface of a lesion prior to cleansing with saline. The Z-stroke technique involves cleaning the wound with saline followed by taking the swab and using a zigzag motion covering the area of interest (eg, intact skin vs lesion) from top to bottom while rotating the swab in a circular motion to ensure even coverage of all swab surfaces. The Levine technique involves rotating a culture swab in a 1-cm^2 area for 5 to 10 seconds with enough pressure to elicit wound fluid from viable tissue in the center of the lesion.[43] Although the Levine technique has primarily been used in chronic wounds, the method has also been used to collect bacterial specimens from intact and nonintact skin using NGS methods.[37] Scientists should use the Levine technique to collect optimal swab specimens. Using cultivation-based methods such as CFUs, Gardner et al[43] compared the results of swab specimen with tissue specimen in chronic wounds. The Levine technique had the highest sensitivity (0.90) and specificity (0.57) for measuring BL when compared with various critical thresholds (such as 1×10^6 CFUs) across the sampling techniques.[37] The study findings suggest that the Levine technique may be the most effective swabbing technique, yet further study needs to be conducted to determine the influence of swabbing technique on BB values when assayed by NGS methods. The consistent use of a validated bacterial specimen technique and the awareness of conditions or substances that may influence the bacterial specimen are essential for ensuring specimen integrity.

Alternately, tissue-specimen collection is necessary when a deeper specimen is required. This allows for the analysis of BB in the dermis and subcutis,[1] which may be of interest for those interested in the BB of anaerobic infections.[44] Depending on the research question, the scientist may want to distinguish the differences in BB on the wound surface (eg, critical colonization) versus deeper tissue involvement (eg, infection). Tissue specimens require a 4- to 5-mm punch biopsy or a scalpel blade to excise a similarly sized tissue sample.[44] For example, using NGS methods, Sprockett et al[37] demonstrated that swab specimens taken before wound debridement provided comparable BL and bacterial diversity values in comparison with the debrided tissue. Therefore, swab and tissue specimens provide comparable measurement of BB; the less invasive and cheaper specimen collection methods, such as swab, may be used in place of tissue biopsy for cutaneous or wound BB analysis.[37]

LANDMARK 5: BACTERIAL SPECIMEN INTERFERING SUBSTANCES

Next-generation sequencing is a sensitive tool that will measure any DNA fragments matching the targeted region that may be present in a sample without regard to the source of the DNA. It is for this reason that extra care must be taken to ameliorate the potential for any DNA contamination of sample materials; poor antiseptic handling techniques and improper sterilization of materials are of primary concern.[33,45] In addition, sampling of nonviable tissue (eg, slough) or wound exudate, collecting the specimen from a suboptimal location, and cleansing the wound with antiseptic before sample collection will confound a sample set. Contamination is likely to result in the overestimation or underestimation of the specimen BB.

When collecting a specimen from a lesion or wound site with nonviable, necrotic tissue (ie, eschar, slough), the specimen may overrepresent bacteria at the site and may confound findings regarding the association between BB and the disease state being evaluated.[37] Bacteria utilize nonviable tissue in the wound bed as a food source to increase proliferation, which subsequently increases the BL.[46] If viable tissue is insufficient to swab, debridement of the nonviable tissue is warranted.[43] The site of specimen collection may underestimate or overestimate the BB in a specimen. For example, Sprockett et al[37] showed that BL was greater in the wound center than the wound edge. Therefore, a swab specimen of the center of the wound likely provides the best characterization of BB in wounds. No additional studies were identified in the literature that evaluated the differences in

BB results as a result of different intralesion or wound specimen harvest sites (eg, center vs edge). Thus, the use of the Levine technique will support optimal specimen collection.[43] Lastly, skin lesions or wounds should not have antiseptics used within 24 hours of specimen collection to avoid altering the bacterial community structure.[24] Conservative recommendations encourage the use of sterile saline to cleanse the wound with sterile gauze before specimen collection to assist with removing excess exudate and, if necessary, to support obtaining an optimal and representative sample of skin surface and wound BB.[45]

LANDMARK 6: SPECIMEN HANDLING, TRANSPORT, AND STORAGE

Specimen handling and transport practices vary in the literature. Regardless of the approach selected, maintaining consistent conditions across specimen handling, transport, and storage is essential to preserve specimen integrity.[6] If temperature conditions are inconsistent, freeze/thaw cycles will occur and result in loss of specimen integrity.[6] Once specimens are collected, conservative recommendations suggest that the specimen should be kept cold or frozen, if the specimen is not moved to long-term refrigerated laboratory storage less than 2 hours following specimen collection.[6,45] As discussed in landmark 2, it is important to monitor the temperature of the samples while in transport and temporary storage.

The influence of different transportation practices on bacterial specimen integrity was not identified in the literature. The standard practice for bacterial swab specimen storage for NGS is in a -80° C refrigerator. Moistened skin swab specimens kept at room temperature over 2 weeks had no difference in bacterial community structure results when compared with samples stored at -20° C.[6] However, best practices suggest that specimens should be maintained at -80° C to ensure that DNA degradation does not occur.[1]

LANDMARK 7: PERFORMING NEXT-GENERATION SEQUENCING OF CUTANEOUS 16S RDNA SPECIES

Most NGS platform manufacturers offer a wide selection of preformulated kits with the decision support resources necessary to identify sample-specific optimized methods and streamline the sequencing process. Should the scientist opt not to use a preformulated kit, attention must be paid to determining specimen sequencing depth and how much data are required to answer the research question at hand.[7] In the case of NGS, 16s commonly used primers for cutaneous and wound BB are selective for 16S rDNA variable regions 1 to 3 (eg, primers 27F, 534R)[9,20] and variable region 4 (ie, 515F, 806R).[37] The selection of primers for particular variable regions may cause an underestimation or overestimation of a particular bacterial taxon. For example, variable regions 1 to 3 have been shown to be highly similar to whole metagenomics shotgun sequencing, and variable region 4 poorly characterizes skin commensal bacteria.[47] Therefore, prior to amplification of the 16S rDNA, scientists should determine the particular primer specificity. Primer specificity rates may be acquired by calling the manufacturer or through exploratory studies, such as those reported by Caporaso et al[48] and Mao et al.[49] In regard to PCR-based sequencing protocols, the Earth Microbiome Project has existing protocols for carrying out 16S rDNA PCR amplification (www.earthmicrobiome.org).

A review of sequencing platforms used to perform PCR assays, and their correlating sequencing error rates, is beyond the purpose of this review. For more details regarding the common sequencer platforms used today, the authors direct those who seek more technical and exhaustive reviews of the topic to Frey et al,[50] Nelson et al,[51] and Nakamura et al.[52] Following selection of the appropriate platform to carry out the PCR assay, the scientist must determine the appropriate 16S rDNA variable region, primers, and sequencing depth.[7]

LANDMARK 8: ANALYTICAL METHODS

Depending on the number of samples and depth of sequencing, the PCR assay output can provide more than 15 million sequences. The amount of data that are produced creates statistical, bioinformatic, and computational challenges.[6] Fortunately, many sequencing platforms provide scientists with analysis tools (eg, Shannon index, UniFrac distance metric) specific to their preformulated kits and workflow. These platform-specific tools are intended to work seamlessly with the data generated and may be a suitable alternative to other open source programs, such as Quantitative Insights Into Microbial Ecology, Mothur, and the Ribosome Database Project, which are available to assist with analysis of amplified 16S rDNA data. Computational programs assist with conducting statistical analysis, determining bacterial community diversity information, and developing publication quality data figures/tables for visual analysis. For more information regarding the use of the above computational methods for data analysis, the authors refer the reader to more detailed articles, such as Goodrich et al[6] and Hamady and Knight.[7] Following completion of the study, authors are encouraged to submit their study data (eg, sequences and covariates) to the International Nucleotide Sequence Database Collaboration to support future BB meta-analysis–type studies.

LANDMARK 9: LIMITATIONS OF NEXT-GENERATION SEQUENCING

Using the 16S rDNA gene for cutaneous and wound BB studies has limitations. Measurement of 16S rDNA cannot differentiate between viable and nonviable bacteria, and the influence of nonviable bacteria on disease processes has not been determined.[10] Sequencing 16S rDNA for BB research elucidates the bacterial community structure, but not what the organisms within the community are doing or how they interact with each other within the context of the disease. In addition, to determine species-level data, longer sequence reads (>700 base pairs) via the Sanger method are required.[10] Although the technology is continually evolving and species-level data may soon be possible, current NGS platforms produce shorter sequence reads of 200 to 600 base pairs (genus-level data). This impedes the ability of scientists to reliably determine bacterial species-level data from NGS sequencing.[7] Therefore, scientists must determine the length of sequence reads and the best method (eg, Sanger vs PCR) to answer the research question.[7]

FUTURE DIRECTIONS

With the advances in technological innovations over the past decade, clinical scientists have increased capacity to characterize the role of bacterial community membership structure (BB) and function in the context of disease. Further technological advances will allow scientists to reliably identify organisms at the species level, specific DNA coding regions of interest, signaling molecules (eg, those used in quorum sensing), and transfer of plasmids or other genetic material shared among community members. Currently, if a clinical scientist is interested in such things and wants to characterize the phylogenetic bacterial community structure and function, whole-genome shotgun metagenomic sequencing should be performed. For a more in-depth review of metagenomics, see Weinstock.[53]

For example, the 2 common bacterial phenotypes found in medical and natural environments are free-living (planktonic) and the more structured biofilm formation; both are capable of interrupting homeostasis.[54] In the context of microbiome research, metagenomics allows for the identification of bacterial functions that lead to biofilm formation. Whole-genome shotgun metagenomic sequencing encompasses the combined use of bioinformatics, metatranscriptomics, proteomics, metabolomics, and sequencing of the 16S rDNA[55]; such studies provide a more comprehensive view of the influence of bacteria within their environment. Future interdisciplinary investigations that combine biomarkers with metagenomic data will provide the most comprehensive evaluation of BB's influence on cutaneous and wound disease outcomes. Determining the influence of psychological stress on cutaneous BB signatures and existing host environment (eg, leg) physiological changes (eg, impaired tissue perfusion, excessive tissue edema) of arterial, venous, and/or diabetic wounds is one of many potential uses for NGS technologies.

CONCLUSIONS

Currently, there is a paucity of clinician-driven BB research using NGS. In this article, the authors present considerations that clinical scientists must address prior to initiating BB research. As the overall cost of NGS sequencing has decreased and the capacity of individual laboratories to perform molecular techniques and analysis increases, clinician scientists have an abundance of opportunities to conduct interdisciplinary BB research. With ongoing attention to appropriate bacterial specimen collection procedures, collection materials, specimen storage, and specimen processing (eg, PCR assays), scientists can ensure maximal reliability and validity of the data. As a result, the high-quality data will support the identification of transformative information, which will advance scientific attempts to support precision management of cutaneous and wound disease processes by developing BB interventions targeted at the bacterial community (eg, commensal bacteria). For example, identifying ways to leverage the relationship with commensal bacteria will allow scientists to better understand lingering questions regarding public health concerns, such as the impact of widespread antiseptic use and its role on BB associated with community-acquired infections. In NGS technology, scientists have access to a powerful technique that allows specific and sensitive mapping of the interactions between a host and its microbiome. In this manner, BB may be used as a tool to identify the overall influence of systemic disease processes or the role in the development of cutaneous and wound diseases in the context of longitudinal studies.[27]

As the body of data grows, the ability to characterize dynamic interactions of individual and community bacterial species with the host that prevent or cause cutaneous or wound disease development will be enhanced. This will lead to the development of targeted microbiome diagnostics and therapeutics to restore regional BB stability.

PRACTICE PEARLS

- Bacterial bioburden is operationalized into 3 biomarkers: (1) the total quantity of bacteria present, or bacterial load; (2) the number of distinct bacterial taxa present in a specimen, known as bacterial diversity; and (3) the existence of pathogenic organisms in the skin/wound environment.[9]

• Next-generation DNA sequencing, also known as deep sequencing or pyrosequencing, has enhanced researchers' capacity to identify, characterize, and elucidate the commensal or pathological role that bacteria play in human immunity, disease, and metabolic processes via the amplification of genetic material.
• The NGS-based characterization of bacterial communities utilizes the 16S rRNA gene. The 16S rRNA gene encodes RNA that is part of the bacterial 30S small subunit ribosome, and contains 9 hypervariable regions that can be sequenced to determine specific bacterial species (operational taxonomic unit)
• Variability in BB is greatly influenced by biogeography, environment, and individual host factors, each of which needs to be considered and measured or controlled to ensure accurate interpretation of the BB.
• Collecting a bacterial specimen from nonviable tissue, wound exudate, suboptimal location, and cleansing the wound with antiseptic prior to sample collection will confound a sample set.
• The amount of data that is produced from NGS creates statistical, bioinformatic, and computational challenges. In preparing to conduct BB research, building an interdisciplinary research team (with bioinformaticians and medical microbiologists) is necessary to support successful projects.

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