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ORIGINAL ARTICLE

Fungal Nails? DNA Facts Challenge Dystrophic Etiology

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Historically recalcitrant to treatment, infection of the nail unit is a pervasive clinical condition affecting about 10%-20% of the U.S. population; patients present with both cosmetic symptomatology and pain, with subsequent dystrophic morphology. To date, the presumptive infectious etiologies include classically-reported fungal dermatophytes, non-dermatophyte molds, and yeasts. Until now, the prevalence and potential contribution of bacteria to the

clinical course of dystrophic nails had been relatively overlooked, if not dismissed. Previously, diagnosis had been largely made via clinical presentation, although microscopic examinations (KOH) of nail scrapings to identify fungal agents, and more recently, panel-specific PCR assays have been employed to elucidate causative infectious agents. Each of these tools suffers from test-specific limitations. However, molecular-age medicine now includes DNA-based tools to universally assess any microbe or pathogen with a known DNA sequence. This affords clinicians with rapid DNA sequencing technologies at their disposal. These sequencing-based diagnostic tools confer the accuracy of DNA level certainty, while concurrently obviating cultivation or microbial phenotypical biases. Using DNA sequencing-based diagnostics, the results herein document the first identification and quantification of significant bacterial, rather than mycotic, pathogens to the clinical manifestation of dystrophic nails. In direct opposition to the prevailing and presumptive mycotic-based etiologies, the results herein invoke questions about the very basis for our current standards of care, including effective treatment regimens.

In the clinical setting, onychomycosis is the predominant, presumptive diagnosis for thickened and dystrophic nails, which affects 10%-20% of the U.S. population.[1,2] Due to the high prevalence, refractory nature of the condition, high rates of treatment failures, and recurrence, the condition has frustrated both patients and the medical community for decades.[3] Dermatophytes, such as *Trichophyton rubrum* and other mentagrophytes, are considered the

primary contributing agents,[4-9] however, non-dermatophytic fungal contributors have also been reported at an approximate prevalence of up to 18%.[8,10-15] Further, positive identification frequencies of *T. rubrum* and *T. interdigitale* were previously found to be approximately 30% when direct nail mycological exam showed the presence of fungal agents.[16-19] These data support administration of antifungals as first-line therapy, however treatment failure rates are high, prompting prolonged, systemic antifungal administration, which carries, in some cases, risk of severe hepatotoxicity.[20] In addition, comorbid conditions such as diabetes mellitus, advanced age, and repetitive micro-trauma may further confound effectiveness of dystrophic nail treatment.[21-22] Taken collectively, these findings warrant additional investigation into identifying the agent(s) responsible for manifestation of dystrophic nails.

In spite of the prevalence of dystrophic nails, the unequivocal diagnosis of the underlying cause remains elusive. The use of microscopic and culture-based methods have contributed little to increasing the incidence of positive treatment outcomes. For example, until recently, the most common laboratory diagnostic approach utilized microscopic analysis via potassium hydroxide, returning false positives rates up to 15%.[23] Alternatively, traditional culturing methodologies, the current standard of care for bacterial assessment, suffer from very high false negatives for fungal identification due to poor growth in a variety of media.[24] Although histological examination and periodic acid-Schiff (PAS) outcomes can identify the presence of fungus, speciation is limited and these tools do not elucidate or consider contributions from bacterial

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pathogens.[24] Finally, well-established molecular methods, such as polymerase chain reaction (PCR)-based platforms, offer rapid and accurate diagnostic information. They are, however, generally limited to identification of only a handful of species, and mandate predetermination within an *a priori* testing panel.[16-19,25] In contrast to these commonly-used approaches, more recent, rapid, next-generation DNA sequencing methods are not limited to a predetermined "panel" of targets, but rather have the ability to universally identify microbes, without limiting analysis to a narrow subset of suspected pathogens. This advantage dramatically expands identification capability with a greater degree of comprehensive accuracy.[26-27] Due to copy number variations amongst species, the 16*S* control is not perfect, however, it is accepted as an appropriate control for next-generation DNA sequencing. Thus, this study was undertaken to investigate and achieve a comprehensive evaluation of the true microbial census found in dystrophic nail specimens, using comprehensive next-generation DNA sequencing technologies as the method of detection. In so doing, the authors explore alternative potential pathogenic etiologies and clinical considerations of the medical sequelae within the diagnosis of "dystrophic" or "fungal" nails.

Materials and Methods

In accordance with protocols approved by The Institutional Review Board of South University, in Savannah, Georgia, a total of 8958 nail specimens, one from each of 8958 patients with

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clinically suspect and symptomatic nail pathology, and 16 healthy, control specimens, one from each of 16 healthy patients, were obtained from patients at multiple clinics across the United States, utilizing the MicroGen DX (Lubbock, TX) protocol. To avoid confounding the data with inadvertent cross-contamination, only a single specimen (control or dystrophic) was collected from each patient.

Nails were examined for suspected onychomycosis with dystrophic and hypertrophied nails. Classic findings included thickened, discolored, detached nail plates with subungual debris. Nails were first cleaned of all polish and/or creams. An alcohol swab was then utilized to grossly remove local surface contaminants. A sterile nail cutter was employed to obtain a full-thickness clipping of nail from the most proximal aspect of the unattached plate. In addition, a sterile curette was used to remove subungual debris; both nail and debris were carefully placed in a sterile dry specimen tube and bag for transport and to avoid environmental contamination. Control specimens were obtained in an identical fashion from normal volunteers who displayed healthy nail appearance, devoid of dystrophic characteristics. Patient specimens were then submitted to MicroGen DX for analysis using molecular diagnostic methods, namely extraction of microbial DNA, amplification by PCR, tag encoding of molecular components, and subsequent determination of bacterial and fungal species using next-generation DNA sequencing.

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Results

Patients with visible dystrophic pathology presented to their physicians seeking treatment, subsequently submitting specimens for next-generation DNA sequencing. Of the 8958 dystrophic specimens analyzed, 8863 also disclosed information on age and gender. Of these 8863 dystrophic specimens, 5366, or 60.5%, were from female patients; 3497, or 39.5%, were from male patients. These data suggest that females had about 1.5 times higher likelihood of seeking treatment for dystrophic nails compared to males. The average age of female subjects was 57.5 years; the average of male subjects was 57.4 years. As shown in Figure 1, the highest percentage of specimens submitted was in the 61-70 years of age group at 23.84%; the next highest percentage occurred in the 51-60 years of age group, at 19.24%. The next highest percentage occurred in the 71-80 years of age group, at 16.76%. The distribution of specimens amongst 10-year age groups, as a percentage of the total for each gender, was virtually identical. For example, 23.63% (1268 of 5366) of female specimens originated from subjects between 61 and 70 years of age and 24.16% (845 of 3497) of male specimens originated from subjects in this same age group. In comparing the percentages of specimens analyzed in each of the ten 10-year age groups, no discernible distinctions were observed between genders.

Importantly, of the dystrophic specimens clinically suspected of onychomycosis, only 49.5% (4433 of 8958) contained one or more fungal species. Within these 4433 mycotic-positive specimens, a diverse census of 1387 different fungal species were identified. Of the identified

fungal species, the ten most prevalent species within the control and dystrophic nail specimens are presented in Figure 2. The most prevalent dermatophyte/fungal species included *Trichophyton rubrum* (19.3%), *Pithomyces chartarum* (9.9%), and *Epicoccum nigrum* (8.7%). By comparison, fungus was not present in any control specimens.

As expected, bacteria were identified in 100% of the control specimens. The ten most prevalent species in control and dystrophic nail specimens are depicted in Figure 3. *Corynebacterium tuberculostericum* was the most prevalent species, detected in 75% of the control specimens. *Staphylococcus epidermidis* and *Staphylococcus capitis* were detected at more modest prevalence (56.3% and 25.0%, respectively) in the control specimens. Contrary to conventional paradigms, bacteria were also identified in 100% of the 8958 dystrophic nail specimens, including those devoid of mycotic contribution. Each dystrophic specimen tested positive for one or more bacterial species, with 986 different species identified. *Staphylococcus epidermidis* was the most prevalent species, detected in 64.4% of all dystrophic nail specimens; *Staphylococcus pettenkoferi* (49.4%) and *Corynebacterium tuberculostearicum* (35.1%) were also frequently detected.

Though bacteria were present in both control and dystrophic specimens, the distribution of species was significantly different. An analysis of means demonstrated a greater abundance in the dystrophic specimens compared to controls. The average bacterial concentration in control specimens was 36,975 cfu/gm, while the mean of the dystrophic specimens (n = 8958) was

3,185,455 cfu/gm. Thus, dystrophic specimens' average bacterial concentration was ~86 times higher compared to normal controls. Further, 83.2% (4 out of 5) of dystrophic specimens had counts greater than 10^5 while only 18.7% (less than 1 out of 5) of normal controls reached that level of microbial abundance. In agreement with these values, the average bacterial concentration of the dystrophic specimens analyzed herein ranged from 10^5-10^7 cfu/gm. Bacteria were further categorized based on broad phenotypical classifications such as Gram staining and oxygen requirements. Of the ten most prevalent bacterial species in dystrophic nails, nine were Gram-positive and either aerobic or facultative. *Staphylococcus epidermidis* (64.4%), *Staphylococcus pettenkoferi* (49.4%), and *Corynebacterium tuberculostearicum* (35.1%) were among the ten most prevalent Gram-positive, aerobic or facultative species identified, as presented in Table 1. In addition, *Moraxella osloensis* (2.5%), *Pseudomonas aeruginosa* (2.0%), and *Stenotrophomonas maltophilia* (1.7%) were among the five most prevalent Gram-negative, aerobic or facultative species identified, as presented in Table 2. Interestingly, while the prevalence of gram-negative bacteria in these specimens is relatively "rare", when identified their numbers appear to have a tendency toward significance. For example, the prevalence of *Pseudomonas aeruginosa* is only 2%, however, when present, its average contribution is a more notable 30%. Finally, among the five most prevalent anaerobic species, all were Gram-positive. *Anaerococcus octavius* (8.4%), *Propionibacterium acnes* (2.7%), and *Anaerococcus hydrogenalis* (2.3%) were noted, as presented in Table 3.

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Discussion

Infections of the nail plate, with associated dystrophic changes, are a prevalent clinical issue. To date, dermatophytes have been presumed the predominant infectious contributor. Failure to address bacteria as significant pathogenic contributors of dystrophic nails, however, may account for poor treatment responses and the high rate of recurrence associated with current therapeutic interventions. In addition, prolonged reliance on antifungals, in attempts to eradicate recalcitrant infections that are not mycotic driven, may contribute to unnecessary hepatic exposure and/or hepatotoxicity. Additionally, although contributions such as advanced age, repeated micro-trauma, peripheral vascular disease, and diabetes are often acknowledged in treating dystrophic nails, the bacterial contribution to dystrophic etiology has not received the same consideration in academic or clinical settings. Ultimately, the lack of objective and accurate diagnostic laboratory testing has stymied treatment of dystrophic nails.

Next-generation DNA sequencing methods offer rapid, objective, sensitive, and accurate methods to comprehensively characterize diverse microbial censuses. Using this comprehensive pathogen diagnostic, our analysis of 8958 dystrophic specimens showed that only 49.5% were positive for a dermatophyte or fungus. Further, *T. rubrum* prevalence, previously reported to range from 70%-90%, was identified in this study at a profoundly lower rate, 19.3%. *Pithomyces chartarum* was the second most prevalent fungus, present at

approximately half (9.9%) that of *T. rubrum*. The next most prevalent dermatophyte reported in the literature, *T. interdigitale,* ranked only 17th among the fungal species reported in our study with a prevalence of merely 2.4%. Interestingly, of the ten most prevalent fungal species reported in our study, nine were non-dermatophyte phenotypes. Specifically, the top ten most prevalent fungal species in the specimens accounted for 71.7% of all fungal positive specimens; 52.4% of the fungal positive specimens contained non-dermatophyte species. Taken together, the specimens demonstrated significant diversity in the fungal and mold species identified herein, rather than a census dominated by dermatophytes as classically reported. These findings are significant and substantially different from characterizations found in the literature, most of which are derived without the benefit of molecular analysis sensitivity or the large sample size leveraged in this survey.

In addition to the fungal results above, and perhaps more important clinically, the results presented herein suggest that bacteria are a significant contributor to the clinical condition of dystrophic nails often reported as “fungal nails”. Treatment consequences aside, molecular analysis demonstrated that bacteria were present in 100% of the dystrophic nail specimens, while only 49.5% of these specimens reported any fungal species. In addition, the distribution and concentration of bacteria among dystrophic specimens, as compared to normal control specimens, were congruent with clinical infection and/or pathology. Anecdotal observation supports a 2-3 log difference in bacterial concentration between “healthy” and “dystrophic”

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nails, as reflected in one of three bacterial abundances: $<10^5$ cfu/gm, 10^5-10^7 cfu/gm, $>10^7$ cfu/gm. In our study, the geomean of the control specimens (n = 16) was ~3.2 x 10^4 cfu/gm, while the mean of the dystrophic specimens (n = 8958) was ~3.2 x 10^6 cfu/gm. Hence, within the dystrophic specimens these organisms have achieved a concentration that is considered, in practice, a clinical infection in the presence of altered physiology.[27] In alignment, we propose the increased concentration of opportunistic organisms, such as *Corynebacterium* and *Staphylococcus* species, may contribute to the manifestation and presentation of dystrophic nails.

Among the dystrophic specimens, the most common bacterial species were overwhelmingly Gram-positive, aerobic, or facultative. Specifically, the top ten species included six *Staphylococcus* species (*epidermidis*- 64.4%, *pettenkoferi*- 49.4%, *lugdunensis*-23.9%, *piscifermentans*- 14.7%, *simulans*- 11.7%, *warneri*- 11.0%), two *Corynebacterium* species (*tuberculostearicum*- 35.1%, *massiliense*- 9.1%), one *Rothia* species (*dentocariosa*- 15.8%), and a single Gram-positive, anaerobic species, *Anaerococcus octavius* (8.4%). Of the top ten species, the relative contribution ranges from 6.5% (*A. octavius*) to 40% (*S. pettenkoferi*). Among Gram-positive aerobic or facultative species, the average relative contribution ranged from 6.6% (*C. aurimucosium*) to 40% (*S. pettenkoferi*). Also, of particular note were the prevalence and contributions of Gram-negative aerobic or facultative species. The most common Gram-negative species had a prevalence of only 1.5%-2.5% (*Enhydrobacter*

aerosaccus, Moraxella osloensis); however, when present, the Gram negative mean relative contribution rose to as high as 30% (range of 7%-30%). Similarly, obligate anaerobes modestly contributed to the census: the most common anaerobic species had a prevalence of 1.1%-8.4% (*Peptoniphilus ivorii, A. octavius*), with a mean relative contribution ranging from 5.6%-6.8% (*A. hydrogenalis, Propionibacterium acnes*). Collectively, our results demonstrate that dystrophic specimens are typically polymicrobial, including some constituents of the healthy skin microbiome, potentially complicating clinical progression and treatment.

The fact that only half of the dystrophic nail specimens reported any fungal constituents gives rise to several clinical quandaries, challenging the classic etiology in a chicken vs. egg impasse. It is well known that for at least some clinical cases of dystrophic nails, antifungal therapy alone is effective. This fact would apparently contradict a logical paradigm, which might be drawn based on the present study, where microtrauma engenders bacterial infestation, which provides for opportunistic fungal pathogens. Interestingly, this observation might be explained by parallel findings that antifungals also have antibacterial effects. More specifically, it is known that itraconazole interferes with *Staphylococcus aureus* biofilm formation[28]. As *S. epidermidis*, found at relatively high prevalence in dystrophic specimens, also has an impressive capacity for biofilm formation[29], it is plausible that such bacterial contribution may be mitigated via itraconazole's previously described antibacterial effects, possibly obviating the need for antibacterial therapy. Additional study and analyses are warranted regarding antifungal

therapy in dystrophic specimens to further elucidate and contrast antibacterial from antifungal effects in this important clinical scenario.

Interestingly, Gram-positive bacteria, including *Corynebacterium* and *Staphylococcus* species, predominated in both control and dystrophic nail specimens. We therefore propose that these may be inductive pathogenic microbes that, given opportunity by breaches of the host defense (microtrauma), lead to manifestation of dystrophic nails. First, consistent with observations in bacterial composition in diabetic foot ulcers and in contralateral intact skin[30], the dystrophic nail specimens contain a far higher prevalence and abundance of opportunistic pathogens, such as *Corynebacterium* and various *Staphylococcus* species, as compared to control specimens. For example, *S. epidermidis* is a constituent of healthy skin microbiome[31], but it can become an opportunistic pathogen and has an exceptional capacity to form biofilms[29] and evade both innate and adaptive immunity.[32-33] In addition, *S. epidermidis* is often encountered in chronic infection[30], and may induce interleukin-1-mediated subacute inflammation in infected keratinocytes[34], the predominate cell type generating the nail bed matrix. *S. epidermidis*-induced inflammation may alter keratin architecture, leading to dystrophic manifestations such as crumbling, brittle nails, akin to psoriatic plaques that arise from aberrant keratinocyte proliferation and function in psoriasis. Our data indicated a higher prevalence of *S. epidermidis* in dystrophic specimens (64.4%) compared to control (56.3%). Taken together, *S. epidermidis* may be a likely bacterial candidate that shifts from commensal to opportunistic, infects

keratinocytes, creates biofilms that may shield both bacterial and fungal denizens from treatment, ultimately yielding dystrophic manifestations.

Finally, repeated microtrauma, age, diabetes, vascular compromise, or altered physiology due to immune status provide opportunity to compromise the integrity of the epidermal and nail surfaces, permitting such organisms to become established beyond "contamination" contributing to the pathogenic sequelae. In the authors' experience, presence of these same bacteria in full-thickness chronic wounds impedes healing, especially when normal host defenses are disrupted via physiological and/or environmental factors that support chronicity, including the natural adoption of biofilm phenotypes. Hence, we conclude that opportunistic bacterial denizens may become pathogenic independent of mycotic contribution, leading to manifestation of dystrophic nails. Comprehensive identification of the full microbial census may be pivotal for targeted and/or comprehensive therapy.

In spite of the interesting observations and hypotheses proposed herein, the study includes two limitations: 1) patients' dystrophic nail treatment histories were not collected, and 2) patients' medical histories were not collected. With regard to patients' dystrophic nail treatment histories, the intent of the study was to determine the microbial census based on visual observation of dystrophic nails. In other words, patients were seeking treatment for dystrophic nails, regardless of prior exposure to antibacterial and/or antifungal therapy. The current study provides a foundation for analyzing a patient's microbial census and tailoring subsequent

therapy accordingly. With regard to patients' medical histories, the dearth of this information stymies efforts to correlate etiology and manifestation of dystrophic nails based on microtrauma, diabetes, or other contributing factors. Therefore, it is difficult to ascertain if there is any correlation between morbidity and the microbial census. In spite of this limitation, the data suggest that a higher percentage of individuals seeking treatment were beyond 51 years old, with the highest percentage in the 61-70 age group. Because we did not observe any gender-related changes in the percentages of submitted specimens, we do not suspect that female or male hormones contribute to dystrophic etiology. Therefore, one possible hypothesis for the observation that a higher percentage of specimens are submitted from patients 51 years and older is that patients suffer microtrauma and/or complications from poorly-managed diabetes (as discussed previously), which allows commensal microbes to become opportunistic over time. In other words, our data may suggest a time and opportunity chronicity associated with dystrophic manifestation such that as patients age, they are more likely to develop dystrophic nails and seek treatment. In spite of these limitations, this manuscript offers a promising new strategy for analyzing dystrophic nails and designing improved therapeutic strategies, and subsequent strategies shall explore the hypotheses proposed herein.

Conclusions

Inarguably, diagnosis and treatment of dystrophic nails is a highly variable and poorly understood clinical challenge. Historically, the infectious etiology implicated in dystrophic nails has been indicated as mycotic, to a larger degree, dermatophytes, and to a lesser degree, molds. To date, however, lack of accurate and reliable methods of diagnosis has prevented comprehensive elucidation of the full microbial census for consideration as contributors to the pathology of nail dystrophy. The standard accepted methods of microbial determination were relatively subjective and unreliable, with both high false positive and false negative rates.[18-19] In addition, traditional diagnostic methods suffered from cultivation bias, preferentially identifying microbes that grow well in the particular diagnostic media employed in the analysis. Furthermore, although early molecular methods based on PCR were free from the limitations of cultivation, PCR can never be comprehensive as the scope of identification remains considerably restricted to microbes within each PCR "panel" (chosen as "likely suspects" and subject to human bias). As a result, any bacterial contribution to manifestation of dystrophic nails has been largely occult and therefore predictably given little consideration.

The diagnostic tool leveraged in this study, next-generation DNA sequencing, is a universal diagnostic method, requiring no *a priori* presumption of the microbes present. Simply stated, if microbial DNA, whether bacterial, fungal, or dermatophyte is present, it can be sequenced and identified without suffering from the aforementioned limitations. This comprehensive

approach dramatically increases clinical elucidation of the microbial census found in dystrophic nail specimens.

Profoundly, the data presented herein from nearly 9000 dystrophic specimens suggest that bacteria, not fungi, are the most prevalent organisms within dystrophic nails. Further considering the abundance of bacteria identified in dystrophic specimens (vs. normal controls), these collective findings challenge the presumption that fungal pathogens are the primary genera responsible for the dystrophic nail cascade. Our data support the hypothesis that bacteria are previously unrecognized and therefore underappreciated contributors to dystrophic nails. In addition, in contrast to the current, widely-held clinical assumption, *Trichophyton rubrum* was present in only about 10% of dystrophic nail specimens. With this broader understanding of the vast diversity of both bacterial and fungal opportunistic microbes so prevalent in dystrophic nail specimens, the authors propose that more targeted, specific, and comprehensive treatment protocols be evaluated in a controlled clinical context. The authors further propose that targeted treatment, with antibacterial drugs alone, or in concert with antifungals, as dictated by each patient's specific microbial constituents quantified by molecular methods, will improve clinical outcomes. Ultimately, rapid and accurate elucidation of the comprehensive microbial census offers a wealth of knowledge and new perspective for clinical consideration in the diagnosis and subsequent treatment of historically recalcitrant dystrophic nails.

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Table 1. Most prevalent Gram-positive aerobic or facultative species in dystrophic nail specimens and average contribution to total census

	Prevalence	Average Contribution
Species	**(% of 8958)**	**(%) to Total Census**
Staphylococcus epidermidis	64.4	27.5
Staphylococcus pettenkoferi	49.4	40.5
Corynebacterium tuberculostearicum	35.1	11.8
Staphylococcus lugdunensis	23.9	10.2
Rothia dentocariosa	15.8	21.9
Staphylococcus piscifermentans	14.7	21.2
Staphylococcus simulans	11.7	13.8
Staphylococcus warneri	11.0	9.7
Corynebacterium massiliense	9.1	8.3
Corynebacterium aurimucosum	2.5	6.6

Table 2. Five most prevalent Gram-negative aerobic or facultative bacterial species as percentage of total for dystrophic nail specimens, and their average contribution to the total bacterial census.

	Prevalence	Average Contribution
Species	**(% of 8958)**	**(%) to Total Census**
Moraxella osloensis	2.5	7.0
Pseudomonas aeruginosa	2.0	30.0
Stenotrophomonas maltophilia	1.7	13.5
Pseudomonas trivialis	1.6	13.9
Enhydrobacter aerosaccus	1.5	11.4

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Table 3. Five most prevalent anaerobic species as percentage of total for dystrophic nail specimens, and their average contribution to the total bacterial census.

	Prevalence	Average Contribution
Species	**(% of 8958)**	**(%) to Total Census**
Anaerococcus octavius	8.4	6.5
Propionibacterium acnes	2.7	6.8
Anaerococcus hydrogenalis	2.3	5.6
Anaerococcus vaginalis	1.7	6.7
Peptoniphilus ivorii	1.1	6.2

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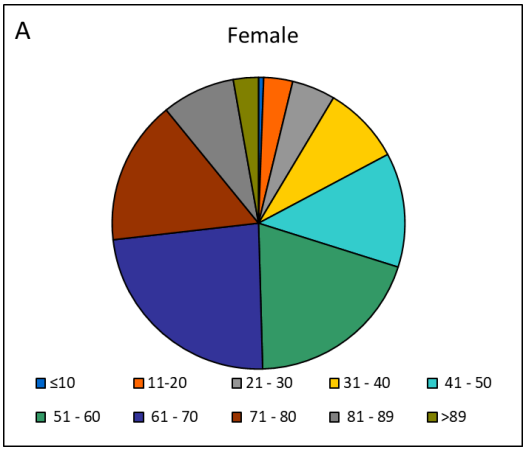
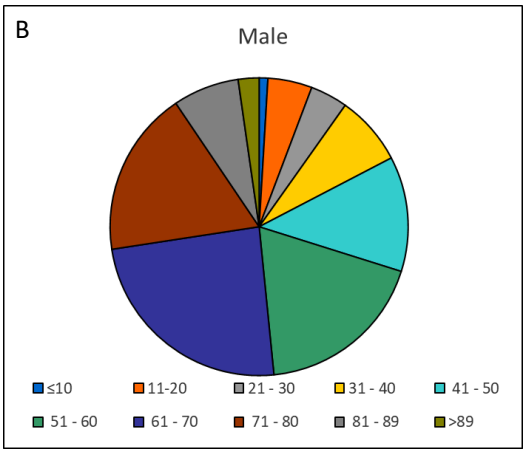
Figure 1. Distribution of age and gender of dystrophic specimens.





Age (yrs)	Female	Male	Sum
≤10	30 (0.56%)	33 (0.94%)	63 (0.71%)
11-20	172 (3.21%)	168 (4.80%)	340 (3.84%)
21-30	257(4.79%)	142 (4.06%)	399 (4.50%)
31-40	464 (8.65%)	264 (7.55%)	728 (8.21%)
41-50	680 (12.67%)	437 (12.50%)	1117 (12.60%)
51-60	1056 (19.68%)	649 (18.56%)	1705 (19.24%)
61-70	1268 (23.63%)	845 (24.16%)	2113 (23.84%)
71-80	856 (15.95%)	629 (17.99%)	1485 (16.76%)
81-89	434 (8.09%)	251 (7.18%)	685 (7.73%)
>89	149 (2.78%)	79 (2.26%)	228 (2.57%)
Specimens:	5366 (60.5%)	3497 (39.5%)	8863 (100%)

Figure 1: Distribution of age and gender of 8863 dystrophic specimens that disclosed information on age and gender.

Panel A: distribution of submitted dystrophic specimens from female patients in each of ten 10-year age groups, as a percentage of total female specimens submitted.

Panel B: distribution of submitted dystrophic specimens from male patients in each of ten 10-year age groups, as a percentage of total male specimens submitted.

Table: 60.5% of submitted specimens were from female subjects; 39.5% were from male subjects. Subjects ages 61 – 70 submitted the highest percentage of specimens,

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at 23.84% of all dystrophic specimens. Of the dystrophic specimens analyzed, the percentage submitted from each 10-year age group was virtually identical amongst female and male specimens. For example, 23.63% (1268 of 5366) of female and 24.16% (845 of 3497) of male specimens were from the 61-70 years age group. In addition, 19.68% (1056 of 5366) of female and 18.56% (649 of 3497) male specimens were from the 51-60 years age group.

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Figure 2. Prevalence of fungal species as a percent of total species in control and dystrophic nail specimens.

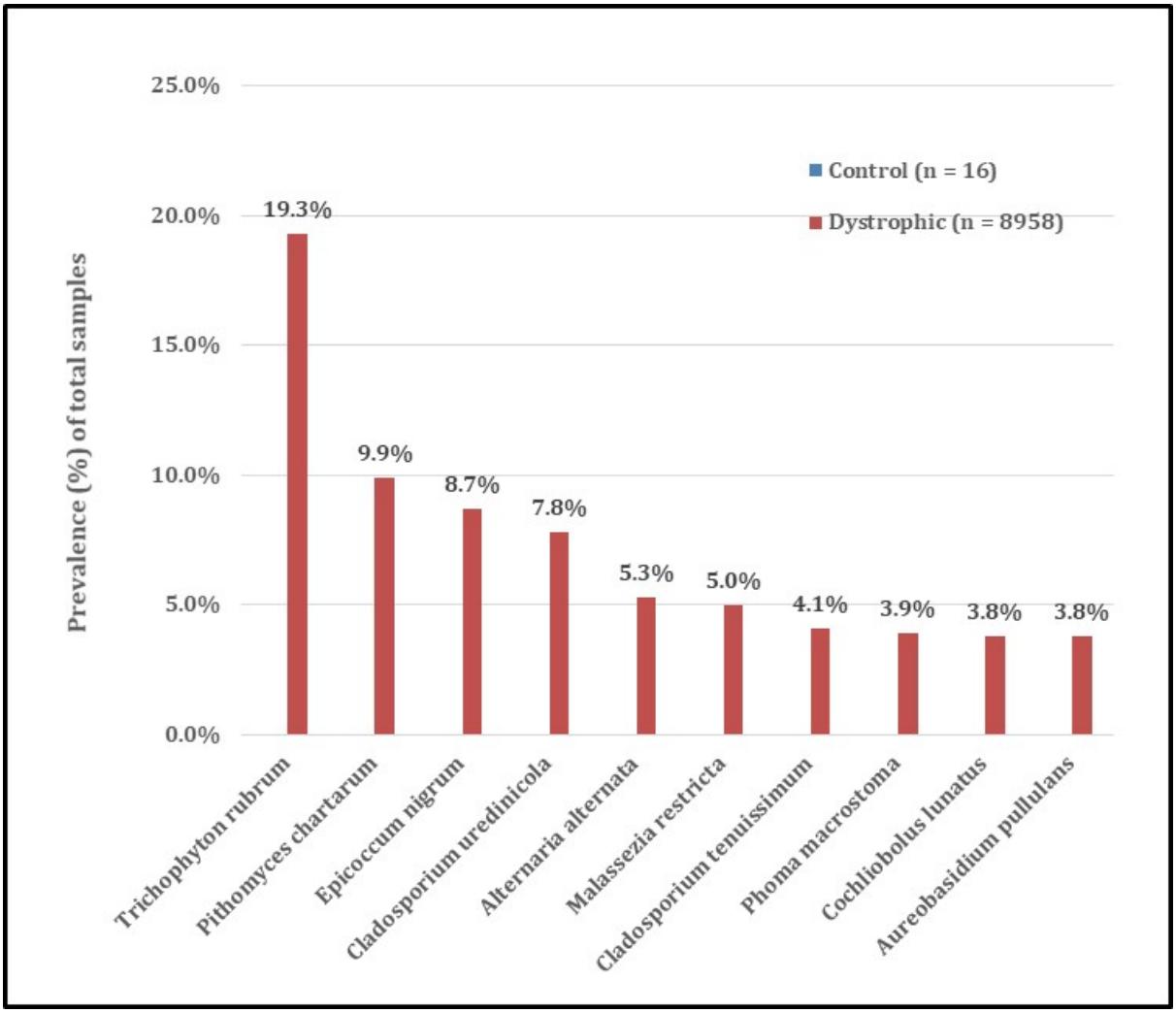

Figure 2: Prevalence of fungal species in control and dystrophic nail specimens. Of the identified fungal species, the ten most prevalent species from each sample is presented. Zero percent of 16 control specimens contained fungal species; 49.5% of 8958 dystrophic nail specimens, or 4433 specimens, contained fungal species.

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Figure 3. Prevalence of bacterial species as a percent of total species in control and dystrophic nail specimens.

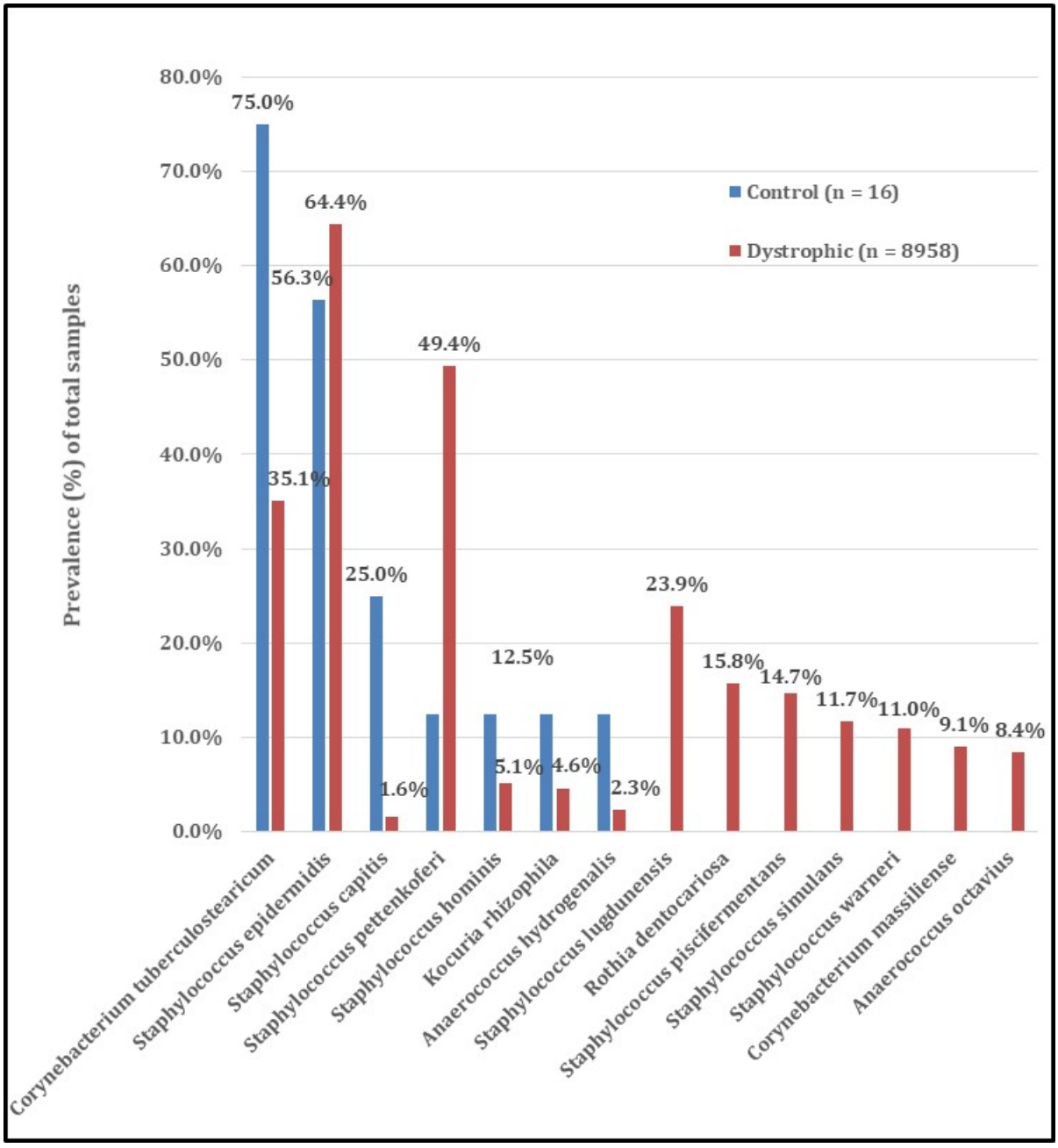

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Figure 3: Prevalence of bacterial species in control and dystrophic nail specimens. Of the identified bacterial species, the ten most prevalent species from each sample is presented. 100% of 16 control specimens contained bacterial species; 100% of 8958 dystrophic nail specimens contained bacterial species.