Bacillus cereus strain isolated from *Demodex folliculorum* in patients with topical steroid-induced rosaceiform facial dermatitis*

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Abstract: The aim of the study was to identify *Bacillus* species from the *Demodex folliculorum* of patients with topical steroid-induced facial rosaceiform dermatitis. Of the 75 patients examined, 20% had clinical spinulosis, while 18.66% had dermoscopic features of *Demodex*: follicular plugs and tails. Of the 17.33% positive patients identified upon microscopy for *Demodex*, samples for bacterial culture were plated on trypticase soy Colombia agar. Identification was performed by microorganisms grown method mass spectrometry. We identified a strain of *Bacillus cereus*.
Keywords: Dermoscopy; Facial dermatoses; Mass spectrometry; Mite infestations; Receptors, steroid; Rosacea

INTRODUCTION

Bacillus oleronius isolated from *Demodex folliculorum* has been identified as a trigger of inflammation in rosacea.[1] Skin lesions associated with an abnormal increase in *Demodex* mites, classified as secondary demodicosis, occur mostly in patients undergoing treatment with topical steroids or calcineurin inhibitors.[2]

The aim of the study was to assess the microbioma in patients with topical steroid-induced facial rosaceiform dermatitis(TSIFRD) and the possible association between *Bacillus* and *Demodex* species.

METHODS

Patients with TSIFRD were included in this study, entailing clinical examination, dermoscopy and microscopy analysis of *Demodex* species. Furthermore, skin biopsies were performed on patients with inflammatory lesions. For the microscopic examination, samples were harvested from the affected area using a piece of Scotch transparent tape measuring 2cm, which was subsequently displayed in a microscope slide and examined under optical microscopy (10x- 40x Zeiss). The sample for bacterial culture was spun two minutes and plated (trypticase soy agar and Columbia agar with 5% sheep's blood (Oxoid – Thermo Fischer, UK), then incubated at 37ºC for 48 hours. Identification was performed by microorganisms grown method MALDI-TOF mass spectrometry (Matrix Assisted Laser Desorption Time-of-Flight ionization - Bruker Daltonik GmbH, Bremen, Germany). Colonies were transferred, emulsified and centrifuged, the supernatant was removed and the residue reconstituted in 70% formic acid. Measurements were performed using MALDI-TOF MS Microflex LT through the FlexControl software. The spectra were collected and analyzed via the Biotyper database (database [DB] -5627 Species list). Scores of above 2 were considered significant for genus and species identification. Mass spectrometry was used to identify the genus and species. The local ethics committee approved the study.

RESULTS

Seventy-five patients with variable TSIFRD subtypes were examined. Dermoscopy revealed: telangiectasias (100% of patients), pustules (80%), scales (54.66%) and atrophy (20%), as white structureless patches between vessels. Clinical spinulosis was found in 15 patients (20%), of which 14-18.66% bore dermoscopic features of *Demodex*: follicular plugs and *Demodex* tails (Figures 1 and 2). A biopsy performed on one spinulosis area showed a histopathologically characteristic image (Figure 3). Thirteen patients (17.33%) were positive for *Demodex* upon microscopy: 8 for *D. folliculorum* and 5

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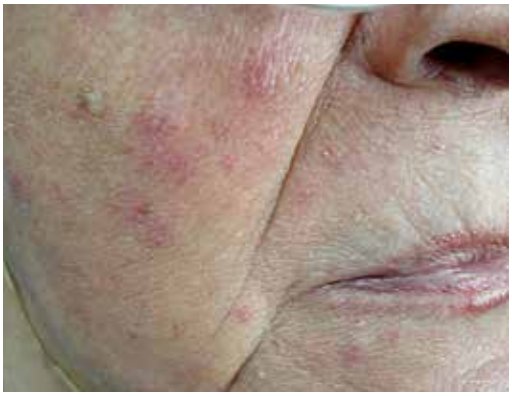

Figure 1: Spinulosis of the face

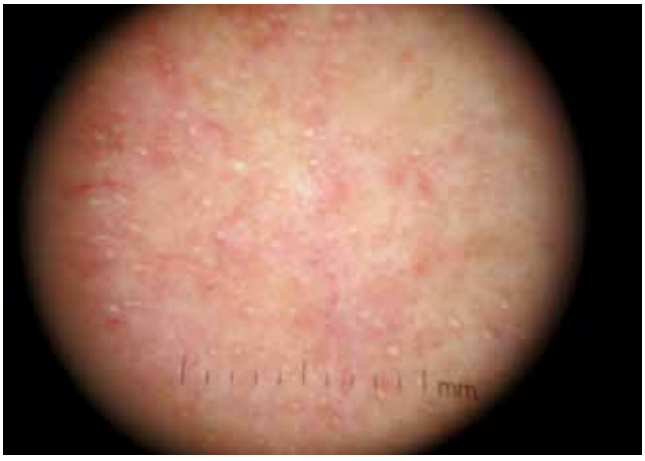

Figure 2: Dermoscopic follicular plugs and *Demodex* tails

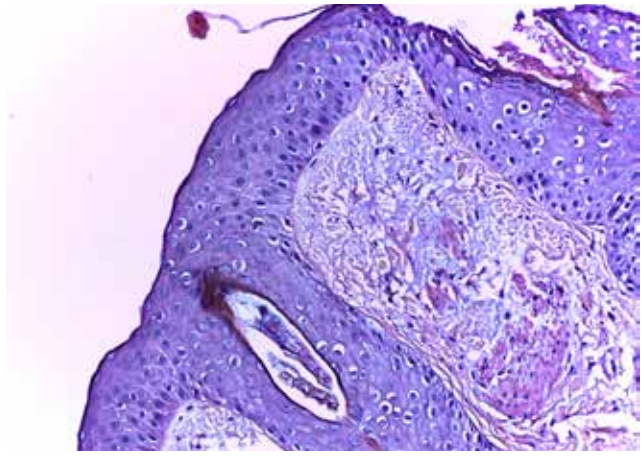

Figure 3: Histopathology of spinulosis revealed *Demodex folliculorum;* hematoxylin/eosine staining,40x

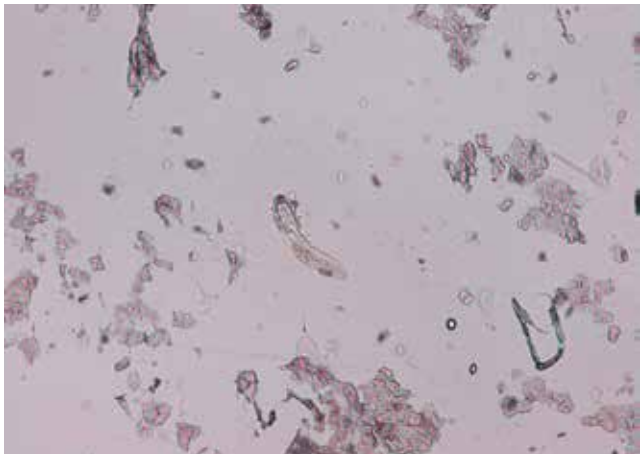

Figure 4: *Demodex folliculorum* smear,10x

Figure 5: *Demodex brevis* smear,20x

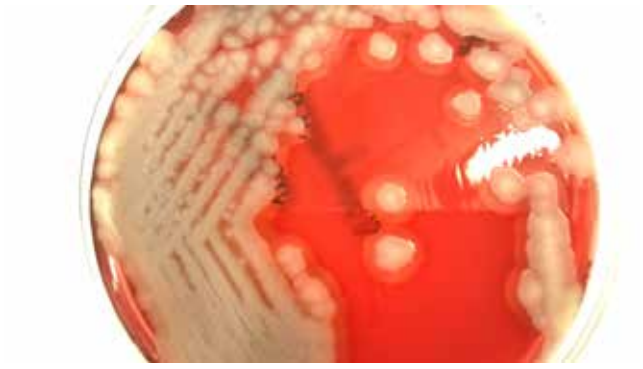

Figure 6: *Bacillus cereus* culture at 72 hours on Colombia agar with 5% sheep's blood

for *D. brevis* (Figures 4 and 5). Among the 8 patients with *D. folliculorum*, one had positive *Bacillus cereus* cultures. No *B. oleronius* or any other bacillus species were found. An analysis of the macroscopic diagnosis of bacterial cultures of the *Bacillus* species oriented morphology *B. cereus* colonies, which were dull gray and opaque, with a rough matted surface.The genus and species were identified by mass spectrometry (Figures 6 and 7).

DISCUSSION

B. oleronius was isolated from only one dissected *Demodex* harvested from one patient with papulopustular rosacea.[3] The absence of serum reactivity to *Bacillus spp.* antigens in 20% of patients with initial rosacea, along with the presence of antibodies in 40% of the controls without visible rosacea (as recorded in previous studies), raises questions about the role of these bacteria as atrig-

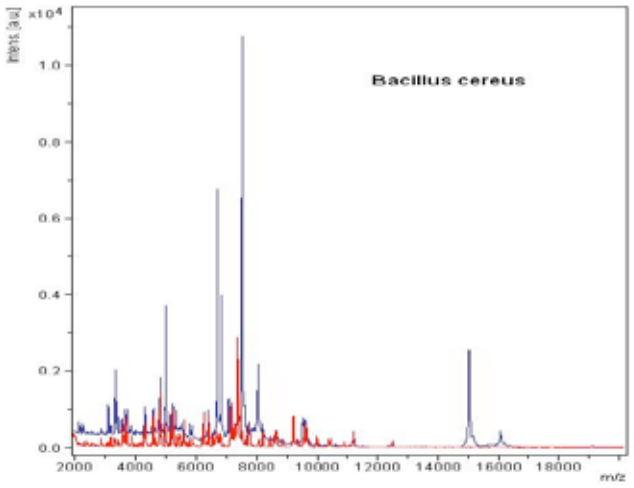

Figure 7: The spectrum obtained from the identification of *Bacillus cereus* by MALDI-TOF spectrometry

ger of the post-steroid rosacea inflammatory process.[4] *B. cereus* is a Gram-positive, aerobic-to-facultative, spore-forming rod widely distributed environmentally, bearing close phenotypic and genetic (16S rRNA) links to several other *Bacillus* species, especially *B. anthracis*.[5] One reservoir for *B. cereus* is the intestinal tract of invertebrates. It has been observed in the guts of certain arthropods which is regarded as the normal intestinal stage in soil-dwelling insects attached to the arthropod intestinal epithelium, where they sporulate. [6,7] In the food industry, *B. cereus* spores are particularly troublesome because spores can be refractory to pasteurization and gamma radiation, while their hydrophobic nature allows them to adhere to surfaces.[8] *B. cereus* spores may colonize skin on the hands and feet, which are often in contact with the environment through microscopic skin abrasions.The pathogenicity of *B. cereus* is intimately associated with tissue-destructive or reactive exoenzyme production: four hemolysins, three phospholipases, one emesis-inducing toxin, and three enterotoxins: hemolysin BL (HBL), nonhemolytic enterotoxin (NHE) and cytotoxin K.[9] In 2008,Dohmae *et al.* described a *B. cereus* nosocomial infection from reused towels in Japan.[10] This study focused on the correlation between *D. folliculorum* and types of *Bacillus* species.The low density of *Demodex* species in the patients included in the study could explain the lack of identification of *B. oleronius* culture, which may underline the difference of microbiome between typical rosacea and TSIFRD.

CONCLUSIONS

In a series of 75 patients with topical steroid-induced facial rosaceiform dermatitis (TSIFRD), 15 had clinical spinulosis, 14 had dermoscopic features of *D. folliculorum* - follicular plugs, *Demodex* tails confirmed by scraping, microscopy and histopathology. Eight out of 15 patients had *D. folliculorum,* and one case revealed associations with positive *B. cereus* cultures; while no *B. oleronius* was isolated. These results show a possible difference between the microbiome found in TSIFRD and previously reported data on microbiome in rosacea. Larger studies are needed to confirm correlations between *Demodex* and *Bacillus* species in different forms of rosacea and their implication in the inflammatory process.❑

REFERENCES

1. Chen W, Plewig G. Human demodicosis: revisit and a proposed classification. Br J Dermatol. 2014;170:1219-25.
2. Antille C, Saurat JH, Lübbe J. Induction of rosaceiform dermatitis during treatment of facial inflammatory dermatoses with tacrolimus ointment. Arch Dermatol. 2004;140:457-60.
3. Lacey N, Ní Raghallaigh S, Powell FC. Demodex mites - commensals,parasites or mutualistic organisms? Dermatology. 2011;222:128-30.
4. O'Reilly N, Menezes N, Kavanagh K. Positive correlation between serum immunoreactivity to Demodex-associated Bacillus proteins and erythematotelangiectatic rosacea. Br J Dermatol. 2012;167:1032-6.
5. Auger S, Ramarao N, Faille C, Fouet A, Aymerich S, Gohar M. Biofilm formation and cell surface properties among pathogenic and nonpathogenic strains of the *Bacillus cereus* group. Appl Environ Microbiol. 2009;75:6616-8.
6. Jensen GB, Hansen BM, Eilenberg J, Mahillon J. The hidden lifestyles of *Bacillus cereus* and relatives. Environ Microbiol. 2003;5:631-40.
7. Margulis L, Jorgensen JZ, Dolan S, Kolchinsky R, Rainey FA, Lo SC. The arthromitus stage of B. cereus: intestinal symbionts of animals. Proc Natl Acad Sci U S A. 1998;95:1236-41.
8. Andersson A, Granum PE, Rönner U. The adhesion of *Bacillus cereus* spores to epithelial cells might be an additional virulence mechanism. Int J Food Microbiol. 1998;39:93-9.
9. Sakai C, Iuchi T, Ishii A, Kumagai K, Takagi T. Bacillus cereus brain abssesses occurring in severely neutropenic patients: successful treatment with antimicrobial agents, granulocyte colony-stimulating factor, and surgical drainage. Intern Med. 2001;40:654-7.
10. Dohmae S, Okubo T, Higuchi W, Takano T, Isobe H, Baranovich T, et al. *Bacillus cereus* nosocomial infection from reused towels in Japan. J Hosp Infect. 2008;69:361-7.

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