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Characterization of Wound Microbes in Epidermolysis Bullosa: Results from the Epidermolysis Bullosa Clinical Characterization and Outcomes Database

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Dr. Lucky has been a consultant and/or investigator for Abeona Therapeutics, Castle Creek Pharmaceuticals, Fibrocell Science, ProQR, and Scioderm.

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Dr. Hook has been a consultant and/or investigator for Amicus/ Scioderm and Castle Creek Pharmaceuticals.

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Abstract

Background/Objectives: Patients with epidermolysis bullosa (EB) require care of wounds that are colonized or infected with bacteria. A subset are at risk for squamous cell carcinoma and bacterial-host interactions have been considered in this risk. The EB Clinical Characterization and Outcomes Database serves as a repository of information from EB patients at multiple centers in

the United States and Canada. Access to this resource enabled broad scale analysis of wound cultures.

Methods: A retrospective analysis of 739 wound cultures from 158 patients from 13 centers between 2001 and 2018.

Results: Of 152 patients with a positive culture, *Staphylococcus aureus* (SA) was recovered from 131 patients (86%), *Pseudomonas aeruginosa* (PA) from 56 (37%) and *Streptococcus pyogenes* (GAS) from 34 (22%). 68% of patients had cultures positive for methicillin-sensitive SA and 47% methicillin-resistant SA (18 patients had cultures that grew both methicillin-susceptible and methicillin-resistant SA at different points in time). Of 15 patients with SA positive cultures with recorded mupirocin susceptibility testing, 11 had mupirocin susceptible SA and 6 patients mupirocin resistant SA (2 patients grew both mupirocin susceptible and resistant SA). SCC was reported in 23 patients in the entire database, of whom 10 had documented wound cultures positive for SA, PA and *Proteus* species in 90%, 50% and 20% of cases, respectively.

Conclusions: SA and PA were the most commonly isolated bacteria from wounds. Methicillin and mupirocin resistance were reported in 47% and 40% of patients tested, respectively, highlighting the importance of ongoing antimicrobial strategies to limit antibiotic resistance.

Keywords

Epidermolysis Bullosa; Wound; Cultures; Microbes; Resistance

Introduction

Patients with epidermolysis bullosa (EB) require ongoing care of wounds that are often colonized or infected with bacteria. A subset of EB patients are at risk for squamous cell carcinoma (SCC) and certain bacterial-host interactions have been speculated to be implicated in this risk.[1–3] In 2016, our group carried out a single center, observational study to characterize wound culture results along with bacterial susceptibilities in this population (a subset of whom are included in this larger cohort). *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA) and *Streptococcus* spp., were the most commonly isolated bacteria. Additional findings included the presence of mupirocin-resistant (MupR) SA isolates in 65% (13/23) of patients.[4]

The EB Clinical Characterization and Outcomes Database (EBCCOD) serves as a repository of information relating to numerous aspects of EB patients from multiple centers spanning the United States and Canada. We sought to further characterize the microbes living on EB patients from wound culture results using this valuable resource. Characterization of wound cultures and susceptibilities, as well as increased knowledge of how patients with EB care for their wounds, helps to identify areas in need of improvement and may optimize antimicrobial treatment recommendations.

Our objectives were to 1) Analyze wound culture results from EB patients on a multicenter level, including mupirocin susceptibilities, when available and 2) Gather pilot data to determine the relationship, if any, between wound culture results and SCC risk.

Materials and Methods:

We conducted a retrospective analysis of 717 patients from the EBCCOD, a Research Electronic Data Capture (REDCap) database maintained at the University of Colorado Denver. In total, 739 wound cultures from 158 patients from 13 centers were recorded in the database and extracted in March 2018 for analysis. (Figure 1) Dates of cultures recorded ranged from 2001 to 2018. We reported descriptive statistics, including counts and proportions. Statistical support was obtained from the Biostatistics Department at Columbia University Irving Medical Center, and all statistical analyses were performed using SAS 9.4.

Results:

Patient Demographics:

158 patients from 13 participating centers had recorded wound cultures. 78 (49%) subjects were female, and 80 (51%) were male. The mean (SD) age was 12.8 years ± 0.5 years (range from 0.1 years to 69.2 years), representing a range of EB subtypes (Table 1).

Wound Culture Characteristics:

158 patients had at least one wound culture recorded in the database. 739 wound culture results were recorded for all patients with a median number of 2 cultures per patient (interquartile range 1–5). Wound cultures included both superficial swab and tissue cultures. Of those wound cultures with a recorded method of collection, 689 were obtained from a swab and 19 were from a tissue.

Wound Characteristics:

90 (57%) of patients had wounds that appeared clinically infected, 64 (40.5%) had wounds that did not appear clinically infected and 4 patients (2.5%) did not have any clinical information recorded. Signs of clinical infection included, “expanding raised borders”, “local pain”, “increased local temperature”, “purulent exudate”, “foul odor” or “other”. Purulent exudate was the most common clinical feature. “Other” signs of infection included, but were not limited to pustules, induration, maceration, chronicity and yellow/honey colored crust. The presence of clinical signs of infection or lack thereof was not temporally correlated with wound culture results as captured in the database.

Wound Culture Results:

Out of 158 patients, 152 had at least one positive culture result. The remaining 6 patients had negative cultures. Of those patients with a positive culture, 131 (86%) were positive for *Staphylococcus aureus* (SA), 56 (37%) for *Pseudomonas aeruginosa* (PA), 34 (22%) for *Streptococcus pyogenes* (GAS), 31 (20%) for *Corynebacterium* spp. and 17 (11%) for *Proteus* spp. (Figure 2). Other bacteria isolated included coagulase-negative staphylococci (7%), *Streptococcus agalactiae* (Group B) (7%), *Serratia marcescens* (5%), *Klebsiella pneumoniae* (3%), *Enterococcus* spp. (4%), *Klebsiella oxytoca* (3%), *Enterobacter* spp. (<3%) and *Acinetobacter* spp. (<3%). 113 out of 158 patients (72%) grew more than 1 bacterium.

Isolate Susceptibilities:

Out of 132 patients, 117 had cultures that grew SA and recorded beta-lactam susceptibility testing; 68% of patients had positive growth of methicillin-susceptible SA (MSSA) and 47% of patients for methicillin-resistant SA (MRSA). Both MSSA and MRSA were grown at different points in time in 18 (15%) patients.

Of 15 patients with reported wound cultures results that included mupirocin susceptibility testing, 11 patients had cultures positive for mupirocin-susceptible (mupS) SA and 6 patients had cultures that were positive for mupirocin-resistant (mupR) SA isolates. Two patients grew both mupS and mupR SA isolates. (Figure 3) Mupirocin resistance, as determined by E-test, was classified as low level (MIC 8–256 μ/mL) and high level (MIC ≥ 512 μ/mL). FDA interpretive criteria were utilized for susceptibility classification. Of those 6 patients who had recorded cultures that grew mupR SA, 50% of those same SA isolates were MRSA.

Wound Cultures & SCC:

SCC was reported in 23 out of 717 patients in the database, 10 of whom had recorded wound cultures. For those 10 patients, SA, PA and *Proteus* spp. were isolated in 90%, 50% and 20% of cases, respectively. Cultures from SCC negative patients (142 remaining patients who had reported wound culture data and did not have a history of SCC) grew SA, PA, and *Proteus* spp. in 83%, 34% and 11% of cases, respectively (Figure 4). The small number of patients who had both a history of SCC and recorded wound culture results precluded comparative statistics.

Discussion:

The vast majority of wound cultures performed on patients were positive for at least one bacterial organism. Consistent with prior studies, we found that numerous organisms, including antibiotic-resistant bacteria, colonize the wounds of patients with EB.[4–6] The bacteria most frequently isolated include SA, PA, GAS and *Corynebacterium* spp. SA was the most common bacterium cultured from wounds of patients with EB and nearly half of patients with SA-positive cultures had MRSA. This is consistent with a metagenome sequencing analysis of wounded skin from recessive dystrophic EB patients, which found significantly reduced microbial diversity and a relative increase in staphyloccocal species.[7] Additional studies support the overrepresentation of SA and have demonstrated that multiple staphylococcal species are present within wounds and may change over time, most likely reflecting the species that are in the patients' immediate vicinity.[8]

Colonization, even without clinical signs of infection, requires careful management and strategies due to the risk of developing antibiotic resistance.[9] Mupirocin (pseudomonic acid A) is a topical antibiotic that prevents select bacterial protein synthesis. It is active against staphylococci, streptococci and certain Gram-negative bacteria and is used to treat skin and soft-tissue infections as well as provide a cornerstone for decolonization regimens of MSSA and MRSA.[10] However, the emergence of mupirocin resistance following its use has been demonstrated and is not specific to EB.[10,11]

Although mupirocin susceptibility testing was only performed at select EBCCOD participating sites, among those patients who did have reported mupirocin susceptibility testing, mupirocin resistance was found to be prevalent.

Strategies to manage Gram-negative and flagellated pathogens also require attention. Hoste *et al.* demonstrated that the use of a broad-spectrum fluoroquinolone, administered orally or topically, decreased skin bacterial burden in wounded mice and correlated with a reduction in wound-induced tumor formation.[3] The same study found that topical narrow-spectrum methicillin treatment did not reduce tumor initiation, implying that limiting exposure to flagellated bacteria may be key to protecting against skin cancer development. The proposed mechanism underlying this bacterial interaction is that bacterial flagellin-induced TLR-5 signaling upregulates the alarmin High Mobility Group Box 1 (HMGB1). These molecular signalling events, demonstrated in a mouse model, are hypothesized to explain a potential link between chronic inflammation and skin cancer in humans. Of note, in the same study, HMGB1 was found to be elevated above baseline in SCC tumors of RDEB patients.[3] Additional studies are needed to determine if wound microbiome interventions inhibit the risk of development of SCC and improve outcomes.

Similarly, knowledge of microbial colonization and progression over time on an age continuum will aid clinical decision-making, including patient directed recommendations. In our cohort, PA was present in the wounds of patients as young as 1 month old, suggesting the presence of these microbes at a young age. Conversely, certain bacteria may be acquired at later ages.

Limitations

Wound characteristics, such as clinical signs of infection or lack thereof, as well as topical product use are entered into the registry separate from wound culture results. Therefore, these data could not be temporally correlated with wound culture results.[12]

The limited number of patients who had both a history of SCC and recorded wound culture results within the registry as well as the prolonged duration to the development of SCC, precluded inclusion of large enough numbers to reliably identify microbes that confer a significant risk for the development of SCC.

Conclusions

Given the hypothesized role of bacteria-induced inflammation in the development of wound-associated SCC, improved understanding of what microbes are colonizing and infecting the wounds of our patients may help to isolate those bacteria that confer additional risk for carcinogenesis and therefore may require earlier, more selective treatment.

Resistance to many systemic and topical antibiotic agents in individuals with EB supports surveillance cultures with routine testing for mupirocin resistance as a means to guide antibiotic stewardship and patient counseling. Examples include, use of antiseptic measures to limit bacterial burden and prevent infection, in particular in a wound known to be colonized with MupR SA. Additionally, if a wound becomes clinically infected and recent

surveillance cultures were positive for MupR SA, it may guide initial antibiotic choice while awaiting updated culture results.

In the future, correlation of certain microbes with clinical features, as well as increased understanding of protective bacteria within the normal skin flora, will further aid these recommendations.

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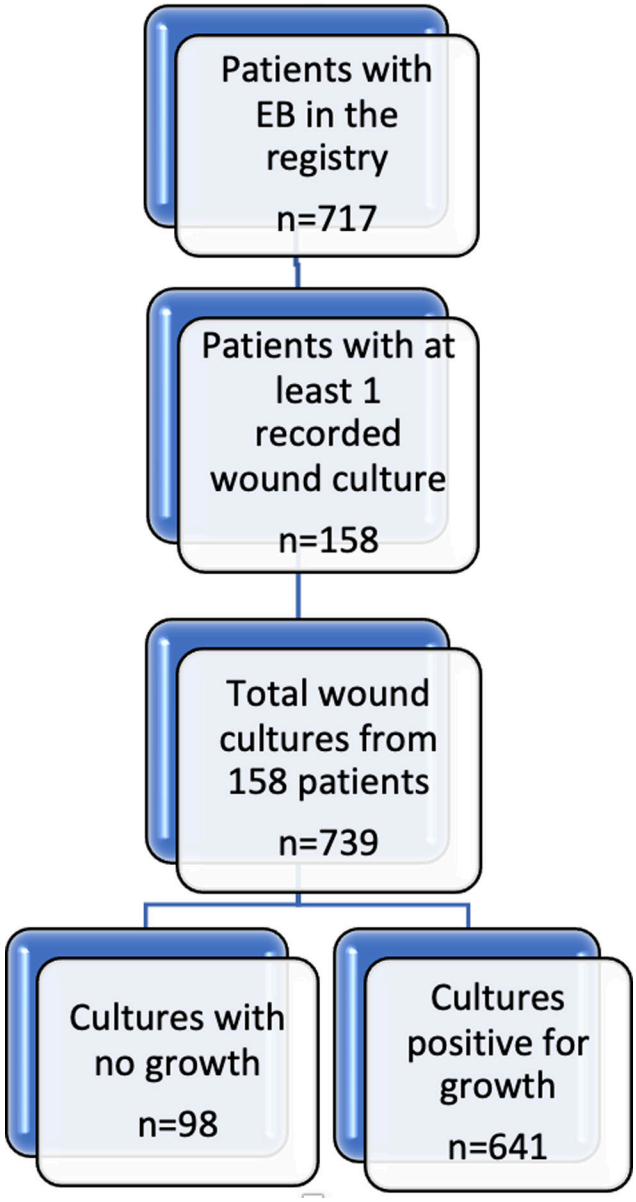

Figure 1:

Flowchart of patient selection and wound culture data extraction: 717 patients with EB were registered in the EB Clinical Characterization and Outcomes Database (EBCCOD) as of March 2018. Of those patients 158 had at least 1 recorded wound culture. In total, 739 wound cultures were recorded from 158 patients. Of those 739 wound cultures, 641 grew at least 1 microbe, whereas 98 resulted in no growth.

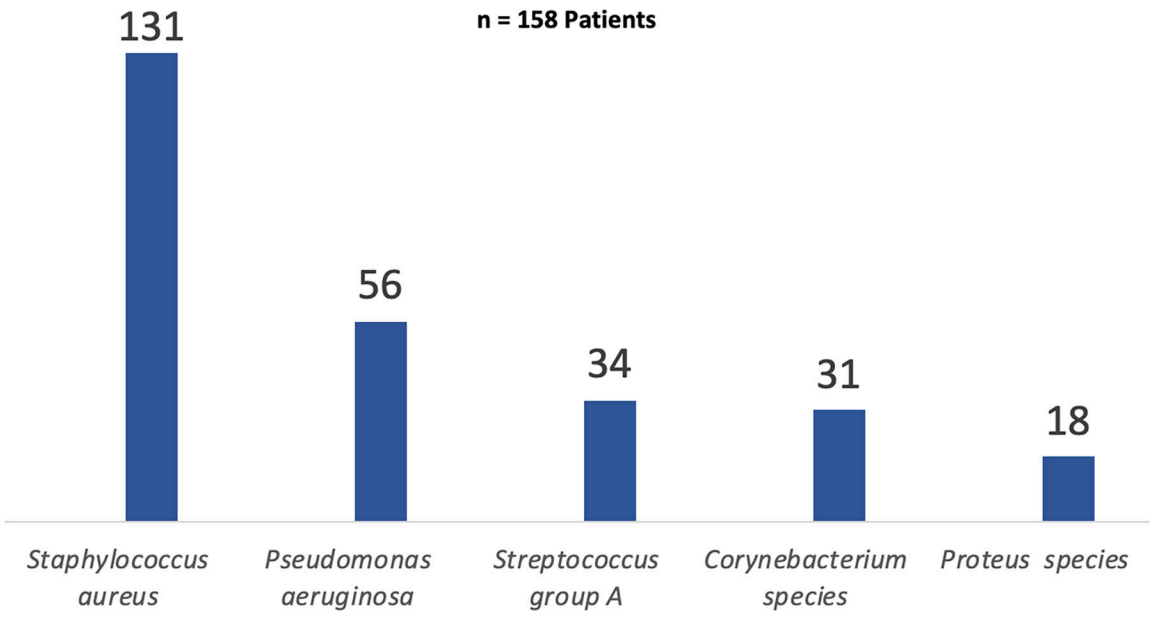

Figure 2:

Most common microbes isolated from wound cultures: Of 158 patients with recorded wound cultures, *Staphylococcus aureus* (SA) grew in 131 (83%), *Pseudomonas aeruginosa* (PA) in 56 (35%), *Streptococcus pyogenes* in 34 (21%), *Corynebacterium* spp. in 31 (19%) and *Proteus* spp. in 18 (11%) patients. Other bacteria included coagulase-negative staphylococci (7%), *Streptococcus agalactiae* (7%), *Serratia marcescens* (5%), *Klebsiella pneumoniae* (4%), *Enterococcus* spp (4%), *Klebsiella oxytoca* (4%), *Enterobacter* spp. (<3%) and *Actinetobacter* spp. (<3%) (data not shown).

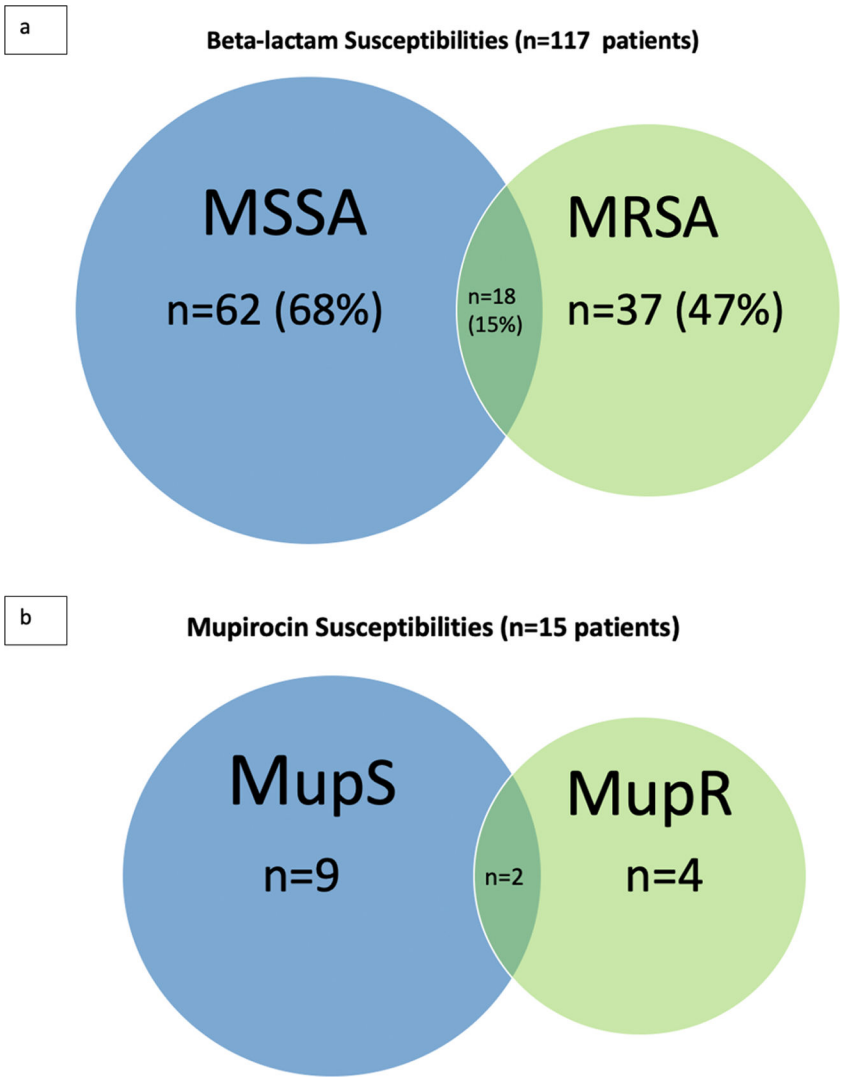

Figure 3:

Beta-lactam and mupirocin susceptibilities: a. Of 117 patients whose wound cultures had recorded SA methicillin-susceptibility testing, 68% of patients had wound cultures growing MSSA and 47% of patients had wound cultures growing MRSA. 18 (15%) patients grew both MSSA and MRSA at different points in time. b. Mupirocin susceptibilities: Of 15 patients whose wound cultures had recorded SA mupirocin susceptibility testing, 11 patients had wound cultures that were mupirocin susceptible (mupS) SA and 6 patients had wound cultures that were mupirocin resistant (mupR) SA isolates. Two patients had both mupS and mupR SA isolates.

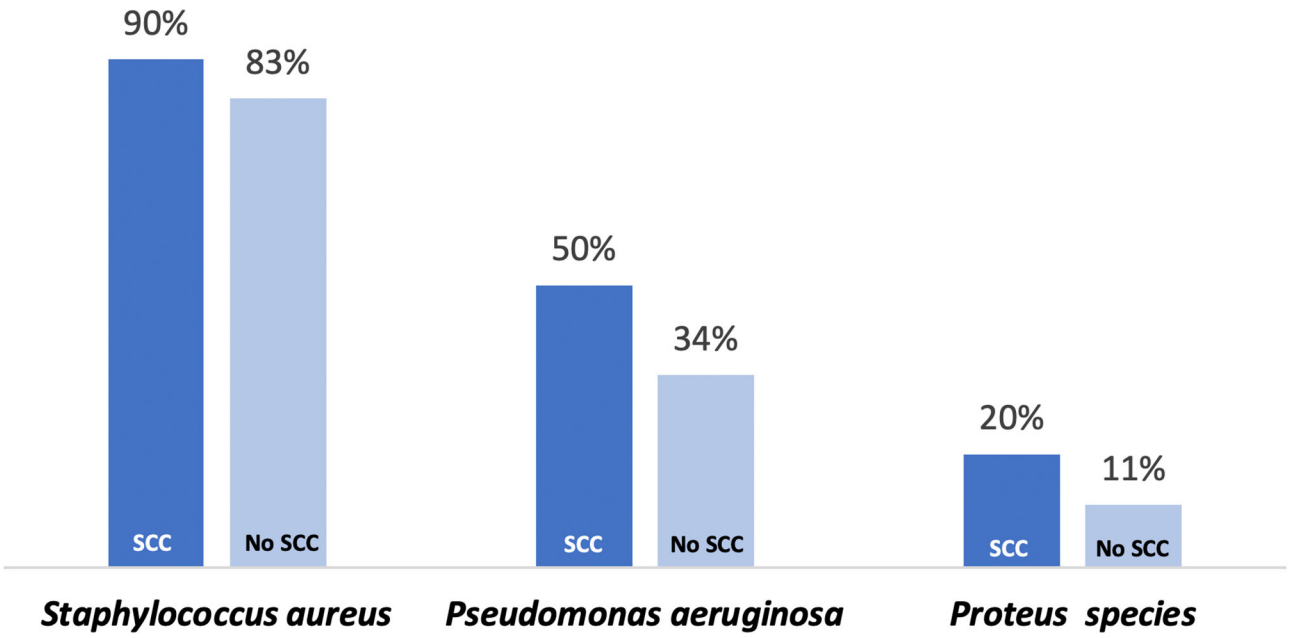

Figure 4:

Percentage of patients with a history of SCC and no history of SCC with positive cultures for *S. aureus* (SA), *Pseudomonas aeruginosa* (PA) and *Proteus* spp. SCC was reported in 23 patients in the database. 10 of these patients also had reported wound culture results. Wound culture results were positive for *S. aureus* (SA), *Pseudomonas aeruginosa* (PA) and *Proteus* spp. in 90%, 50% and 20% of cases, respectively. Cultures from SCC-negative patients (n=142) grew SA, PA, and *Proteus* spp. in 83%, 34% and 11% of cases, respectively.

Table 1:

EB subtypes of the 158 patients with cultures performed.

Diagnosis	n	Percent
Dystrophic (DEB)	**110**	**69.6%**
RDEB	*99*	*90%*
DDEB	*10*	*9%*
Unspecified	*1*	*1%*
EB Simplex	**21**	**13%**
Junctional EB	**22**	**14%**
Unknown	**5**	**3%**
Total	**158**	**100%**

Abbreviations: EB, epidermolysis bullosa; RDEB, recessive dystrophic epidermolysis bullosa; DDEB, dominant dystrophic epidermolysis bullosa.