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Longitudinal study of wound healing status and bacterial colonisation of *Staphylococcus aureus* and *Corynebacterium diphtheriae* in epidermolysis bullosa patients

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Abstract

Epidermolysis bullosa (EB) is an inherited disorder characterised by skin fragility and the appearance of blisters and wounds. Patient wounds are often colonised or infected with bacteria, leading to impaired healing, pain and high risk of death by sepsis. Little is known about the impact of bacterial composition and susceptibility in wound resolution, and there is a need for longitudinal studies to understand healing outcomes with different types of bacterial colonisation. A prospective longitudinal study of 70 wounds from 15 severe EB patients (Junctional and Recessive Dystrophic EB) from Chile. Wounds were selected independently of their infected status. Wound cultures, including bacterial species identification, composition and *Staphylococcus aureus* (SA) antibiotic susceptibility were registered. Wounds were separated into categories according to their healing capacity, recognising chronic, and healing wounds. Hundred-one of the 102 wound cultures were positive for bacterial growth. From these, 100 were SA-positive; 31 were resistant to Ciprofloxacin (31%) and only seven were methicillin-resistant SA (7%). Ciprofloxacin-resistant SA was found significantly predominant in chronic wounds (**$P < .01$). Interestingly, atoxigenic *Corynebacterium diphtheriae* (CD) was identified and found to be the second most abundant recovered bacteria (31/101), present almost always in combination with SA (30/31). CD was only

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found in Recessive Dystrophic EB patients and not related to wound chronicity. Other less frequent bacterial species found included *Pseudomonas aeruginosa*, *Streptococus* spp. and *Proteus* spp. Infection was negatively associated with the healing status of wounds.

KEYWORDS

Corynebacterium diphtheriae, epidermolysis bullosa, skin diseases, *Staphylococcus aureus*, wound healing

Key Messages

- *Staphylococcus aureus* was the most common isolated bacteria from EB wounds, present in 98% of cultures and independently of the wound healing status, EB subtype, patient age, and wound infection status
- Ciprofloxacin-resistant *S. aureus* strains are predominantly found in non-healing wounds
- non-toxigenic strains of *Corynebacterium diphtheriae* were repeatedly found in wounds from Recessive Dystrophic EB patients

1 | INTRODUCTION

Epidermolysis bullosa or EB is a group of rare heritable skin diseases characterised by excessive skin fragility and blisters.[1] EB is caused by mutations in genes coding for structural proteins of the skin, such as type VII collagen, keratin 5 and laminin 332, which severely compromise the integrity and natural barrier of the skin.[2,3] Wound healing is of primary clinical importance in these patient populations. EB patients suffer from repeated cycles of wounding and healing leading to pain, scarring, recurrent infections and even an aggressive type of skin cancer.[4,5]

Two of the most severe forms of EB are Recessive Dystrophic EB (RDEB) and Junctional EB (JEB). These patients present wounds that are slow to heal or become chronic, often remaining open for many years. For these severe EB patients, wounds are especially concerning because they often get colonised or infected with microorganisms, reducing wound healing rates, putting them at risk of death by sepsis and possibly increasing chances to develop secondary squamous cell carcinoma.[6,7,8,9]

Previous studies have shown that wounds in EB patients are mostly colonised by *Staphylococcus aureus* (SA).[10-14] Reimer-Taschenbrecker et al[15] also give evidence for the need to develop longitudinal studies, in order to understand healing outcomes with different types of bacterial colonisation. Only a few studies to date have reported antibiotic susceptibility for EB culture isolates.[10,16-19] For example, for methicillin-resistant SA (MRSA), reports from different countries have shown prevalence ranging from 6.7% to 57%. This considerable MRSA prevalence variation within a rare patient population may be related to a small sample size, but it also highlights possible country-specific intrinsic clinical differences, showing a need for more studies in this field.

Being a rare disease, little is known about the pathways involved in wound chronicity in severe EB.[20-22] Interestingly, in the context of wound healing, there is a high inter and intra-patient variability. Some wounds or areas of the body within an individual patient may experience recurrent wounding and healing and other sites become chronic and stay open for years. Identifying the reasons why certain wounds heal while others do not will thus greatly increase our understanding of EB wound healing, which could certainly contribute to direct clinical management as well as to the discovery of new therapies.

In this regard, a previous study sampling EB patients with and without chronic wounds suggests that SA wound colonisation contributes to the development of chronic wounds in EB patients.[16] However, to date, this observation has not been demonstrated unambiguously. Having a better understanding of the microbial communities affecting these wounds and patients, and their impact in healing, is thus needed to improve current treatments and clinical recommendations.

In this prospective study, our objective was to investigate the role of bacterial composition and SA antibiotic susceptibility for wound healing in the two most severe EB types. To this end, we followed patients and wounds over a 12-month period recording wound healing kinetics, wound infection status, and wound culture results.

2 | MATERIALS AND METHODS

2.1 | Study approval

Informed written consent was obtained from each patient prior to sample collection. This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics committee from Clínica Alemana Universidad del Desarrollo # 2017-85.

2.2 | Patient recruitment

A prospective follow-up study was performed in patients with a confirmed molecular diagnosis of RDEB or JEB. Patient recruitment started in August 2019 and finished with the last patient follow-up in March 2021. All patients were recruited at the study center in Chile (The Epidermolysis Bullosa Research Association [DEBRA] Chile). Initially, each patient was supposed to visit the DEBRA site every 3 months (+/−1 month) and complete a follow-up at 12 months (five visits in total). However, due to the COVID pandemic, patients were only able to complete 1 to 3 site visits and the 12 month follow-up of wound healing kinetics had to be completed by phone call. In total, 10 RDEB patients and five JEB patients were recruited.

2.3 | Wound selection

At the time of patient recruitment (visit 1 or month 0), up to four wounds per patient were sampled, and body location was recorded (head and neck, lower extremities, upper extremities and trunk). To capture all wound types, we selected 1 to 2 *non-healing wounds* (defined as wounds that have been opened for 21 days or more, as indicated by patient; codes 01–02 in Table S1) and 2 to 3 *recent wounds* (defined as wounds that have been open for less than 14 days as indicated by patient; codes 03, 04 and 05 in Table S1). Afterwards, each wound was followed by phone calls and onsite visits for wound closure status recording. At the following visit (visit 2 or month 3), wounds selected at visit 1 were re-evaluated. If any of the wounds had healed and it was not open during the visit, a new wound could be selected for follow-up. If the new wound matched the criteria for a *non-healing wound*, a code 10 was given. If the new wound matched the criteria for a *recent wound*, a code 20, 21 or 22 was given. The same procedure was performed in the following visits until each patient completed a 12 month follow-up. In total, 70 wounds were recruited, 32 of which were recorded as *non-healing*, and 38 as *recent wounds*.

2.4 | Wound healing behaviour and new categories

Besides the initial categories given to the wounds at the time of selection, *non-healing* and *recent*, wounds were further grouped into five distinct categories according to the healing behaviour observed in this study (see Table S1). Category 1, also called 'Chronic', corresponds to wounds that were open for at least 1 year (>52 weeks) at the time of recruitment and continued to be open during the follow-up period. Category 2 corresponds to wounds that were open for at least 1 year (>52 weeks) at the time of recruitment, but closed during the follow-up. Category 3, also called 'Healing', corresponds to wounds that were open for less than 3 months when selected and closed during the follow-up period. After closure, these wounds could have re-opened or remained closed during the follow-up period. Category 4 corresponds to wounds that were open for less than 3 months when selected and remained open during the follow-up period. Finally, Category 5 is composed by wounds that could not be followed up due to patient death (Patient JEB1 died soon after Visit 2 or month 4, see Table S1). From the 70 independent wounds selected, 14 were Category 1 'Chronic', five were Category 2, 47 were Category 3 'Healing', one was Category 4 and three were Category 5. The 'Chronic' and 'Healing' categories were further characterised in this study.

2.5 | Wound Infection status

At patient recruitment, and for every following visit, wounds were evaluated by an infection disease specialist experienced in EB. To assess the wound infection status, we took the MEASURE method into account, creating three novel and distinct categories.[23] Wounds were macroscopically classified as clean, colonised, or infected (done prior to having the bacterial wound culture results). A clean wound was defined as a wound without exudate or serous exudate, with granulation tissue, and either without pain or with very mild pain. A colonised wound or a wound *with critical colonisation* (only mentioned as colonised hereinafter, for simplification) was defined as a wound with a greater amount of exudate, presence of fibrin, moderate pain, and difficulty to heal. An infected wound was defined as a wound with presence of fibrin, with yellowish or purulent exudate, painful, deep in volume, with increased local temperature, bad odour, and erythema around the wound. Infected wounds may have associated systemic symptoms of infection and is likely to be a wound with that does not heal.[6]

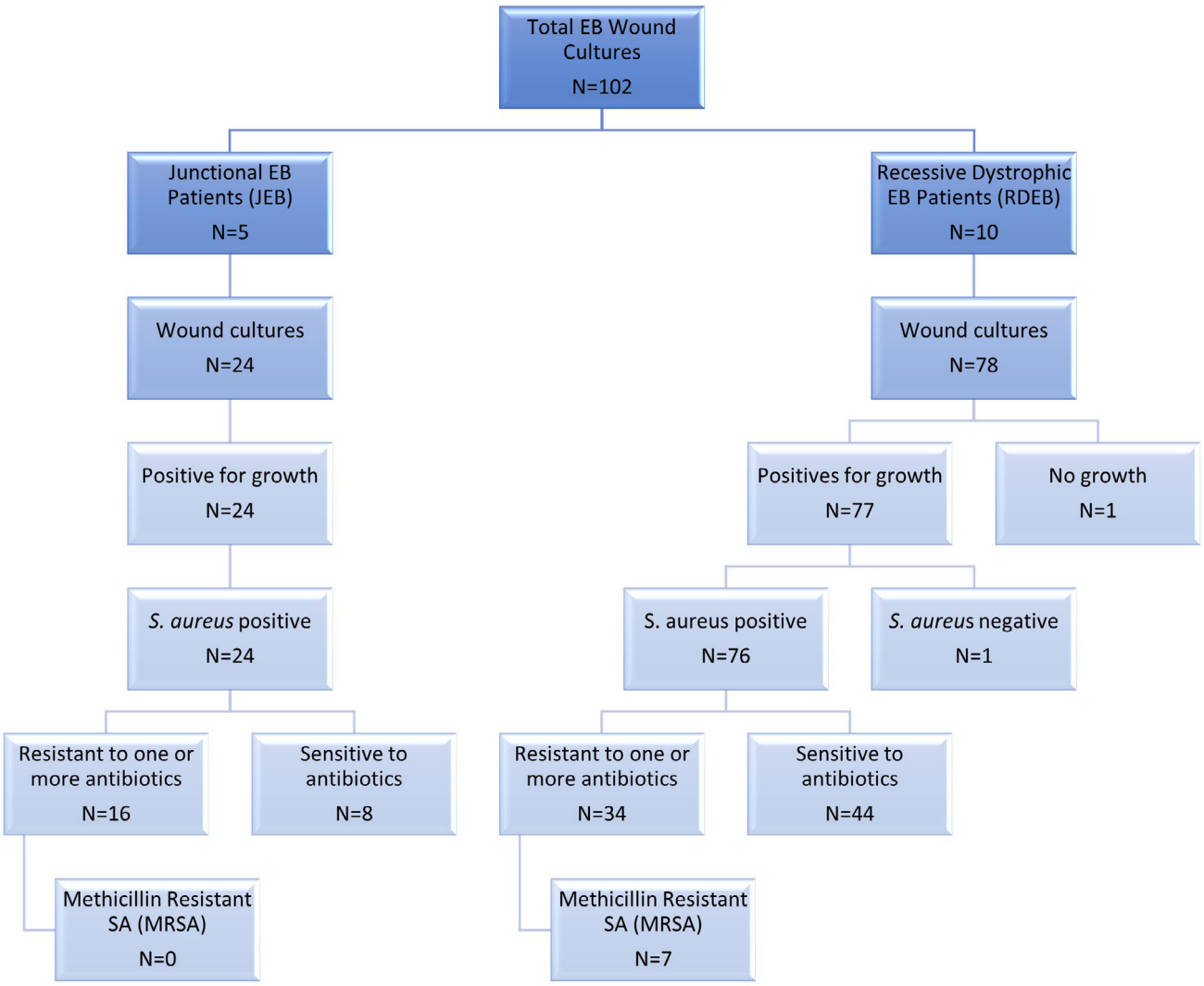

FIGURE 1 Flowchart of patient recruitment, wound culture results, SA presence and antibiotic susceptibility. A total of 102 wound cultures were collected coming from 10 RDEB and 5 JEB patients. From those 102, only one was negative or had no bacterial growth. One hundred were positive for SA (100/102, 98%), and from those 50 were resistant to at least one antibiotic (50/102, 49%). MRSA was only seen in seven wound cultures (7/102, 6.9%) all coming from three RDEB patients (see Table S1)

2.6 | Bacterial wound culture

Each selected wound was cleaned up and debrided prior to collection (this is standard procedure done for EB wounds in a clinical setting when collecting bacterial swabs for microbial determination). Afterwards, a sterile swab tip was gently rubbed against the wound area and place into Stuart media for transportation (T'enT'-SS, Winkler, Chile). The swab was passed in a zig-zag fashion, rotating it 360° to cover the wound bed completely. Samples were taken to the microbiology laboratory at Clínica Alemana, Santiago, Chile, within the same day of sample collection for further analysis. All samples were cultivated on 5% sheep blood agar and MacConkey agar (bioMérieux) and incubated at 35°C for 72 hours. Plates were read every 24 hours. Isolates were identified by mass spectrometry (MALDI-TOF) VITEK MS (bioMérieux). The antimicrobial susceptibility study was performed on the VITEK 2XL (bioMérieux) using AST-P663 card. For *S. aureus* susceptibility, the following antibiotics were tested: Ciprofloxacin, Clindamycin, Cotrimoxazole, Erythromycin, Oxacillin, Rifampicin and Vancomycin.

2.7 | Statistical analysis

Statistical analysis was performed using Prism7 (GraphPad Software, La Jolla, California). The Fisher Exact test was used to compare bacterial composition and susceptibility. The Chi-square test was used to compare wounds' infection status.

3 | RESULTS

3.1 | Patient and wound demographics

A total of 15 EB patients were enrolled in this study, 10 RDEB and 5 JEB patients. Of these 15 patients, nine were female and six were male. The median age of the group was 17 years old, with ages ranging from 0 to 36. Seventy independent wounds were selected and followed. Thirty-two were categorised as *non-healing wounds* and 38 as *recent wounds*. From those 70 wounds, five were located in Head and Neck region, 13 in Trunk, 36 in Upper extremities and 48 in Lower extremities (Table S1).

3.2 | Wound culture results

From the 70 independent wounds selected and followed over time, 102 wound cultures were collected (Figure 1). From those 102, 24 were taken from JEB patients and 78 from RDEB patients. Out of 102 cultures, 101 were positive for bacterial growth (99%) and 100 were positive for *S. aureus* (SA) presence (98%).

Besides SA, other common bacteria found in EB wound cultures were atoxigenic *Corynebacterium diphtheriae* (CD, 31 out of 102 cultures, 30.4%) and *Pseudomonas aeruginosa* (PA, 14 out of 102 cultures, 13.7%) (Figure 2). Interestingly, CD was only found in cultures obtained from RDEB wounds (6 out of 10 RDEB patients, see Table S1).

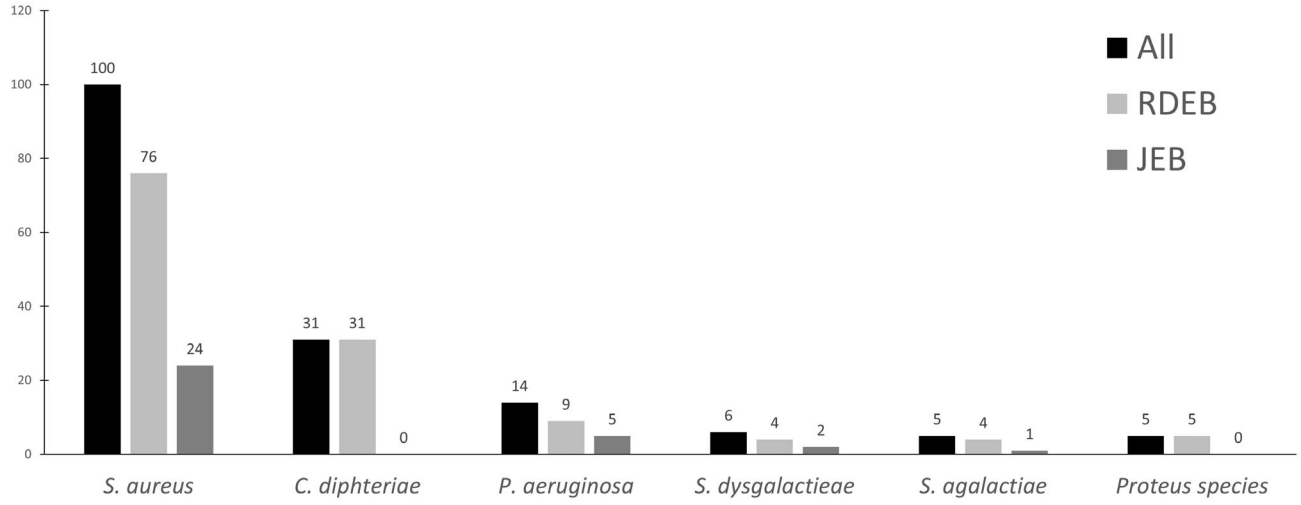

FIGURE 2 Microbial distribution of the most common bacteria isolated from patient wound cultures. Of 102 wound cultures, 100 were positive for S. aureus (SA 98%). The second most common bacterial species was *C. diphtheriae* (CD) found in 30.4% of all cultures, but all present in RDEB patients only (31/78, 39.7%). Other species found were *P. aeruginosa* (PA 13.7%), *S. dysgalactieae* (SD 5.9%), *S. agalacteiae* (SAG 4.9%) and *Proteus* (P 4.9%). Other less frequent bacteria included *E. faecalis* (EF 2.9%), *S. epidermidis* (SE 2.9%), *E. coli* (EC 2%), *S. haemolyticus* (SH <1%), *S. lugdunensis* (SL <1%), *K. pneumoniae* (KP <1%), *S. simulans* (SS <1%) and *S. marcescens* (SM <1%) (data shown in Table S1)

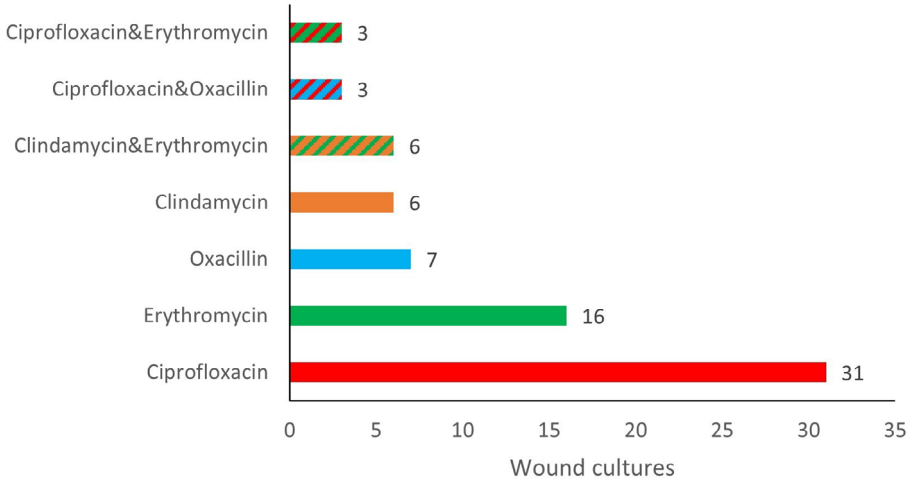

FIGURE 3 Single and double antibiotic resistance from SA positive cultures. From a total of 102 wound cultures and 100 positives for SA, 31 were resistant to Ciprofloxacin, 16 to Erythromycin, 7 to Oxacillin and 6 to Clindamycin. Double resistance was found for Clindamycin and Erythromycin (six cultures), Ciprofloxacin and Oxacillin (three cultures) and Ciprofloxacin and Erythromycin (three cultures)

3.3 | Antibiotic resistance of *S. aureus* isolates

From a total of 100 SA positive cultures, 50 were at least resistant to one antibiotic (50%) and the remaining 50 were susceptible to all antibiotic tested (Table S1). The most prevalent antibiotic resistances were Ciprofloxacin (C, 31/50, 62%), Erythromycin (E, 16/50, 32%), Oxacillin (O, used as proxy for methicillin resistant MRSA, 7/50, 14%) and Clindamycin (Cl, 6/50, 12%) (Figure 3). Double antibiotic resistance was observed in 12 out of the 100 positive SA cultures (12%).

3.4 | Wound infection status

The macroscopic infection status of each wound was recorded during every patient visit, recognising three distinct categories: clean, colonised and infected. From the 102 wound cultures, 102 infection statuses were also collected, of which 17 were clean, 63 were colonised and 22 were infected. These three categories were distributed across all four body sites (with the exception of Head and Neck) and between JEB and RDEB patients (Figure S1A,B).

3.5 | Wound infection status vs *S. aureus* resistance

SA abundance was observed in 98% of wound cultures. From the 100 SA positive cultures, 17 were classified as clean, 61 were colonised and 22 were infected (Table 1).

TABLE 1 Infection status and Ciprofloxacin resistance in *S. aureus* positive cultures[a]

	Total wound cultures	Ciprofloxacin resistant	Susceptible to Ciprofloxacin
Clean	17	4	13
Colonised	61	15	46
Infected	22	12	10

[a]Chi-squared test, *P*-value = .0258*.

From those 100 SA positive cultures, 50 were also resistant to at least one antibiotic (8 were clean, 26 were colonised and 14 were infected). No statistical differences were observed between the infection status and the SA antibiotic resistance to at least one antibiotic. No differences were observed when comparing the infection status and each specific antibiotic susceptibility or double resistance either, exception for Ciprofloxacin (shown in Table 1, *$P < .05$).

3.6 | Wound healing behaviour vs *S. aureus* resistance

Wound cultures coming from 'Chronic' (25 cultures) and 'Healing' (62 cultures) wounds were distributed across all four body sites (with the exception of Head and Neck) and between JEB and RDEB patients (Figure S1C,D).

SA prevalence was observed in 86 out of 87 cultures (98.9%) coming from Chronic and Healing wounds. Only

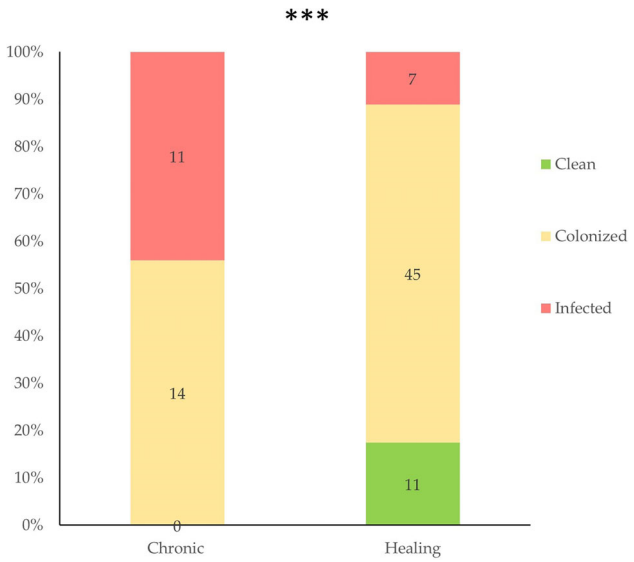

FIGURE 4 Healing status in chronic vs healing wounds. From a total of 25 chronic wounds, 14 were colonised and 14 were infected. From a total of 62 healing wounds, 11 were clean, 45 were colonised and seven were Infected. ***Chi-square test, *P*-value = .0007

TABLE 2 Wound healing status and antibiotic resistance in *S. aureus* positive cultures

	Total wound cultures	Resistant to any antibiotic[a]	Susceptible to any antibiotic[a]	Ciprofloxacin resistant[b]	Susceptible to Ciprofloxacin[b]
Chronic	25	16	9	13	12
Healing	62	23	39	13	49

[a]Fisher exact test, *P*-value = .0318*.
[b]Fisher exact test, *P*-value = .0085**.

one culture from a Healing wound had no bacterial growth (RDEB6-20 month 6, see Table S1).

From 87 wound cultures, 16 and 23 SA were resistant to any antibiotic in Chronic and Healing wounds, respectively (Table 2). This comparison of SA resistance to any antibiotic among Chronic and Healing wounds showed a statically significant difference (*$P < .05$). On the other hand, from the same 87 wound cultures, 13 and 13 SA were resistant to Ciprofloxacin in Chronic and Healing wounds, respectively (Table 2). This comparison of SA resistance to Ciprofloxacin among Chronic and Healing wounds showed a statically significant difference (**$P < .01$).

3.7 | Wound healing behaviour vs infection status

From a total of 87 wound cultures with Healing status, 11 were Clean, 45 were Colonised, 7 were Infected and 14 were Colonised, 11 were Infected in Healing and Chronic wounds, respectively. Healing wounds were significantly less infected than Chronic wounds (Figure 4, $P < .001$***).

4 | DISCUSSION

4.1 | Resource identification initiative

This is the first study in Chile reporting bacterial colonisation and antibiotic resistance in EB patient wounds. We previously reported a pilot study investigating the skin microbiome of patients from Chile and Austria using whole-metagenome sequencing directing from wound swabs, avoiding the need to use culture-dependent methods.[11] In line with that report and many others, we have shown a high prevalence of SA colonisation in EB wounds.[10,24] However, unlike our previous work, here, we have included results from bacterial wound cultures, which are broadly used by clinicians when diagnosis an EB wound infection.

One interesting observation of our study was the presence of SA and particularly antibiotic-resistant SA (to Erythromycin) found in an infant patient (JEB1, Table S1). This patient needed to be repeatedly hospitalised early in life due to the severity of his lesions and unclarity of his EB diagnosis, resulting in patient death at 9 months of age. The first selected wound in this patient was colonised with SA but susceptible to all antibiotic tested. Four months later, another three wounds were collected, and all three cultures showed SA resistance to Erythromycin. Our observation, together with previous reports, suggests there can be microbial colonisation and antibiotic acquisition very early in life, which should be consider by clinicians for patient management and decision-making.[25]

Our study shows that practically all wounds in severe EB Chilean patients are either colonised or infected by bacteria. The most common isolated microorganisms were SA, CD, PA and *Streptococcus* spp. which is consistent with previous studies performed in different settings.[6,10,18,19] Interestingly, from all SA-positive cultures 31% were resistant to Ciprofloxacin and only 7% were MRSA. These results are in contrast with a recent Brazilian study, where they show a lower SA prevalence (51.7%) and higher MRSA frequency (24.7%).[18] These prevalence and antibiotic resistance differences could be due to clinical management, country-specific population, environment and other factors. In Chile, one of the oral antibiotic treatments available at the primary care level are quinolones (such as Ciprofloxacin), widely used for urinary tract infections. Quinolones are not the preferred first-line medication to treat EB skin wounds, but we believe they are being using as such in outpatient units due to clinical ignorance or unavailability of first- or second-generation cephalosporins, amoxicillin/clavulanic acid or trimethoprim sulfamethoxazole, which should be used to treat skin infections. This misuse of quinolones we believe is the main reason to finding such high Ciprofloxacin-resistance SA in our EB patients. It is important to consider these variations in an EB clinical setting especially because MRSA is known to be associated with a higher death rate and with life-threatening complications.[26]

Chronic wound development is common in all subtypes, but particularly more important in the more severe forms (JEB and RDEB) where wounds can be multiple and long-standing, prompting them to bacteria colonisation and ultimately infection.[10] It has also been found that more than 90% of chronic wounds will be colonised with SA at some point of their evolution.[27] One of the main issues regarding prolonged carriage of SA is that it represents a risk factor for resistant strains emerging, probably due to the frequent and sometimes inappropriate use of antibiotics. In fact, MRSA and Ciprofloxacin-resistant *Pseudomonas* are frequently isolated in these patients. This is important since sepsis from cutaneous infection is a frequent cause of morbidity and one of the main causes of death during infancy in severe EB.[9,28]

Another interesting result of our work is that the presence of bacteria in our patients' wounds was not dependent on either their chronicity or infection status. Resistant strains such as MRSA or Ciprofloxacin-resistant SA were more frequently isolated in both chronic and infected wounds, though. These findings may be related to the use of multiple courses of antibiotics. In chronic wounds, there is also an increasing recognition of the so

called concept of 'critical colonisation' as part of the infection continuum, where wound healing may be delayed due to microorganism expansion in the absence of the typical clinical features of infection.[29]

In a clinical setting, only infected wounds are collected via a skin biopsy and microorganisms cultured to find the ones that need to be treated. According to the Infectious Diseases Society of America Guidelines, the gold standard for skin culture in order to differentiate infection from colonisation is tissue culture.[30] This is impractical for EB patients. Apart from the associated costs, the pain and morbidity linked to any skin biopsy collected in EB patients prohibit this plan of action, especially because patients are constantly having wounds with suspicion of infection. Since the clinical distinction between colonisation and infection is difficult, the empirical use of antibiotics in these patients is usual, which must be prescribed according to their local microbiota. It is important to mention the role of topical antimicrobials agents (eg, bleach, vinegar and silver dressings) and topical antibiotics (eg, mupirocin and fusidic acid) for EB wound care.[4] Physicians with experience in EB will certainly avoid the use of systemic antibiotics as much as possible, to reduce the risk of antibiotic resistance. It is thus highly important to perform local bacterial diversity studies where information regarding the most frequent microorganism affecting wounds, and most importantly, the antibiotic-resistance rate is provided. We therefore decided to incorporate wounds from three different categories in the search to understand this bacterial variability.

In this regard, another important finding is the comparatively lower rate of MRSA isolation. Unlike other countries, Chile still has a greater amount of methicillin-susceptible staphylococcus *aureus*, which can be demonstrated by the low circulation of community acquired methicillin-resistant *S. aureus* (MRSA-CA; Information retrieved from the Chilean Ministry of Health).

Regarding the isolation of CD, we presented 31 positive cultures from six patients. CD causes cutaneous diphtheria, and although it is important to notice that all of our CD were toxin negative, this disease can be caused by both toxigenic and non-toxigenic strains. This microorganism has also been described in outbreaks of cutaneous diphtheria among homeless, alcoholic or injection drug using populations.[31,32] To our knowledge, this is the first report showing the specific presence of CD in Epidermolysis bullosa. Previous reports in EB patients have shown presence of other Corynebacterium species from wound cultures, such as *C. striatum*, *C. simulans*, *C. tuberculostearicum* or only reporting presence of Corynebacterium species (or spp).[19,33] Interestingly, the interaction between Corynebacterium species and SA in non-EB conditions has been shown to reduce SA virulence by shifting SA towards commensalism in response to Corynebacterium species.[34] How CD interacts and ultimately influences SA behaviour in EB is largely unknown but warrants further investigation.

Interestingly, in our study, CD was isolated only from RDEB and not JEB patients. Although both subtypes represent the most severe spectrum of the same disease, they differ in the mutated gene causing their EB (JEB and RDEB patients included in this paper have autosomal recessive mutations in the *LAMB3* and the *COL7A1* gene, respectively) and in some extracutaneous manifestations. JEB is also associated with earlier lethality. In our study, the median age was 8 years (range 0-14) for JEB and 20 years (range 2-36) for RDEB. Whether these differences have implications in CD infection rate is largely unknown.

Our work has some limitations. Since EB is a rare disease, the number of patients included in our report is small which may limit the applicability of some of our findings. In this article, we did not include the nutritional status of patients, which is known to modify wound characteristics and healing.[4] However, in our study, we collected healing and non-healing wounds from severe EB patients, thus, our data would show the wound differences that are independent of patient nutritional status. We also do not provide data regarding antibiotic use and duration which may provide additional findings. Our next step is to incorporate that data for further analyses. Lastly, wounds were initially categorised based on patient-delivered information which can be subjected to recall bias.

Finally, our study shows that severe Chilean EB patients are highly colonised with *S. aureus* independently of the wound healing status, EB subtype, age of the patient and infection status of the wound. Antibiotic resistance in SA was shown to have interesting differences to what has been described in other reports and populations, showing a low prevalence of MRSA (7%) and a high frequency of SA resistant to Ciprofloxacin (31%). This should be taken into account when designing further studies and clinical recommendation for the EB community.

Although our study presents results from a small number of EB patients (N = 15), data obtained from all types of wounds, independently of their infection status and healing behaviour, allows us to have a broader overview of this disease and the unbiased contribution of bacterial composition and antibiotic susceptibility on wound healing.

AUTHOR CONTRIBUTIONS

Conceptualization of the study Ignacia Fuentes, María Joao Yubero and Francis Palisson; Methodology Ignacia

Fuentes, María Joao Yubero, Pilar Morandé, Carmen Varela, Lorena Porte; Data analysis Ignacia Fuentes, Esteban Arancibia, Karen Oróstica, Boris Rebolledo-Jaramillo; Project Discussions Ignacia Fuentes, María Joao Yubero, Francis Palisson, Francisco Acevedo; Project administration and funding acquisition Ignacia Fuentes. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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