Bacteria and bioburden and healing in complex wounds: A prognostic systematic review

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Abstract
The wound microbiome may play an important role in the wound healing process. We conducted the first systematic prognosis review investigating whether aspects of the wound microbiome are independent prognostic factors for the healing of complex wounds. We searched Medline, Embase, CINAHL and the Cochrane Library to February 2019. We included longitudinal studies which assessed the independent association of aspects of wound microbiome with healing of complex wounds while controlling for confounding factors. Two reviewers extracted data and assessed risk of bias and certainty of evidence using the GRADE approach. We synthesised studies narratively due to the clinical and methodological heterogeneity of included studies and sparse data. We identified 28 cohorts from 21 studies with a total of 38,604 participants, including people with diabetes and foot ulcers, open surgical wounds, venous leg ulcers and pressure ulcers. Risk of bias varied from low (2 cohorts) to high (17 cohorts); the great majority of participants were in cohorts at high risk of bias. Most evidence related to the association of baseline clinical wound infection with healing. Clinical infection at baseline may be associated with less likelihood of wound healing in foot ulcers in diabetes (HR from cohort with moderate risk of bias 0.53, 95% CI 0.33 to 0.83) or slower healing in open surgical wounds (HR 0.65, 95% CI 0.51 to 0.83); evidence in other wounds is more limited. Most other associations assessed showed no clear relationship with wound healing; evidence was limited and often sparse; and we documented gaps in the evidence. There is low certainty evidence that a diagnosis of wound infection may be prognostic of poorer healing in foot ulcers in diabetes, and some moderate certainty evidence for this in open surgical wounds. Low certainty evidence means that more research could change these findings.

Keywords
bacteria, healing, prognosis
Complex wounds heal by secondary intention—that is by the formation of new tissue rather than by the approximation of the wound edges (primary intention). Complex wounds include pressure, leg, and foot ulcers, and open surgical wounds that, despite different aetiologies, share a common risk of infection and slow healing. These wounds are all usually managed with dressings, although other treatments vary (e.g., venous leg ulcers are treated with compression and pressure ulcers with repositioning and specialist support surfaces).

In 2011, the point prevalence of complex wounds in the United Kingdom was estimated at 1.47 (1.38–1.56) per 1000 of the population, this figure being inferred from a large multiservice cross-sectional study of a single city (Leeds, population c. 750,000). The most frequently documented wounds were pressure ulcers and leg ulcers. Complex wounds take a substantial—but variable—time to heal, impose a considerable burden on people living with them and also have a societal and cost impact in lost activity and medical expenditure. People with complex wounds report that complete wound healing is the most important outcome to them.

There are many potential risk factors or predictors for whether a complex wound will heal and how long this might take. Candidate factors can broadly be grouped as being at: the population level, the individual level, the whole wound level and the cellular level. Prognostic factors at each of these levels are likely to be inter-related in often complex and bidirectional relationships between predictive factors. For example, known factors associated with the future healing of venous leg ulcers include wound size and wound duration at study entry which may also predict pressure ulcer healing. Such factors do not, however, explain much of the observed variation in healing trajectory, and may themselves have a bidirectional relationship with other factors such as the wound microbiome. Elucidating independent relationships between prognostic factors and wound healing requires carefully designed studies which collect a range of clinical, biochemical and microbiological data.

All open wounds contain bacteria: initial colonisation is usually by commensal species from the skin with the potential to acquire or activate virulence factors. Subsequent colonisation of wounds by pathogenic species is also a risk in open wounds. Bacteria are often believed to be present in wounds in the form of biofilms. Biofilms are generally defined as microbial cells surrounded by a polymer matrix of microbial and/or host origin, that adhere to surfaces or to themselves. Growing in this way can make bacteria more tolerant to bactericidal agents.

There are many possible associations between elements of the wound microbiota and wound progress including how long wounds take to heal completely. Suggested prognostic factors include the overall number of microbes (microbial load or bioburden); the specific types of bacteria (including drug-resistant characteristics) in the wound; and/or inter-bacterial interactions and characteristics of resulting biofilms. Most wounds heal despite the presence of microbes so it has been suggested that the balance between bacterial activity and the responsiveness of the host immune system is likely to be of key importance. This develops an earlier concept of critical colonisation, a precursor to clinical infection; this state is often considered, by rather circular reasoning, to be indicated by delayed healing. Diagnosis of infection by contrast usually requires the additional presence of one or more clinical signs such as local pain, heat, redness, swelling and secretion of pus.

Whilst there has been research on the association of the wound microbiome and healing, empirical evidence in this area has not been systematically reviewed. The Australian Wound Management Association states that ‘the true extent of bacterial impairment of wound healing is unknown’. Whilst prognostic association of the microbiome and healing has not been systematically reviewed, there are several treatments available which are suggested to promote healing based on purported antimicrobial activity, for example, silver dressings. Use of these treatments in wounds without clinical infection is predicated on there being a relationship between reducing bacterial load (and perhaps preventing infection) and the wound healing trajectory. Evidence for the effectiveness of these treatments is also unclear. Finding a predictive relationship between aspects of the wound microbiome and wound healing may not necessarily indicate a causal relationship but may, nevertheless, allow better identification of wounds which are likely to show poor healing. Scoping work indicated that the relevant literature was likely to be sparse and diverse, and this supported a decision to adopt broad objectives and inclusion criteria for this review.

### 1.1 Objective

To determine whether aspects of the wound microbiome are independently prognostic for wound healing in people with the following types of complex wounds: pressure ulcers, venous leg ulcers, foot ulcers in people with diabetes, or surgical wounds healing by secondary intention.
controlling for other factors by use of a multivariable analysis or other appropriate method such as the use of propensity matching in the study design. Studies reporting only univariate analyses were noted but not included in the review.

In some cohorts, study authors undertook a multivariable analysis, and applied prespecified criteria for the inclusion of variables in their multivariable analyses. This could mean that the potential bacterial prognostic factor was not included in the final multivariable analysis because its association with healing did not reach criteria for significance—a potentially important finding. We therefore included all studies which carried out multivariable analyses where at least one potential bacterial prognostic factor was considered, including where the variables of interest to our review did not meet the criteria for multivariable analysis. Where this was the case, we noted that variables did not meet criteria for inclusion in the multivariable analysis and, if possible, reported the univariate associations. We considered the appropriateness of the criteria used to determine which variables were included in the multivariable analyses as part of our risk of bias assessment. Similarly, where there were primary outcome data for an association, we noted studies reporting only our secondary outcome of change in wound size, but did not analyse these.

Complex wounds were defined as: foot ulceration in people with diabetes (Wagner grade I or above); venous or mixed aetiology leg ulcers; pressure ulcers (stage II or above); surgical wounds healing by secondary intention (open surgical wounds). We did not further limit eligibility and included studies in which at least 75% of participants had a relevant wound. We excluded studies of burn wounds, which often heal by secondary intention but are outside the scope of this review because of the specific immunological issues associated with burn injuries.

We considered the following types of potential bacterial prognostic factors: Bioburden: Presence or absence of wound infection, however defined; other measures of bioburden including quantification of colony forming units, assessment of critical colonisation, or detectable biofilm measurement. Bacterial typing: Diversity measured by the number of different strains or species; the presence of particular types of bacteria such as Gram-positive or Gram-negative; anaerobic species; the presence of bacteria with characteristics such as multi-drug resistance; the presence of particular strains of bacteria including but not limited to those identified as pathogens. Response to treatment: responses to antimicrobial treatment including changes in any previously specified aspect of the microbiome. We accepted study author definitions of potential prognostic factors because we anticipated substantial heterogeneity in the literature.

Any approach to obtaining samples from wounds and any method of measurement of prognostic factors was permitted, but we excluded measures of bacterial presence taken from extra-wound sources such as blood samples or biopsies from other locations.

### 2.1.1 Outcomes

Primary outcomes were time to wound healing (survival analysis) and proportion of healed wounds at any specified timepoint. An association ratio measure less than 1 indicated a risk of poorer healing (fewer wounds healed or longer time to healing) when the potential prognostic variable was present.

The secondary outcome was the surrogate outcome of change or rate of change in wound size; we planned to analyse these secondary outcome data only if no primary outcome data were available for an association. As our primary outcome was reported for all associations analyses of secondary data are reported in Supporting Information Tables.

### 2.1.2 Search

We searched the following databases in February 2019: Ovid Medline (from 1946), Ovid Embase (from 1946), CINAHL (from 1982) and Cochrane Central Register of Controlled Trials. We also checked the references of included studies and identified systematic reviews. Details of the search strategy are provided in the Appendix.

### 2.1.3 Selection of studies, data extraction and risk of bias and quality assessment

Data were extracted by one review author and checked by a second using a standardised data extraction sheet (piloted on a small number of studies). We extracted data on the following: country and setting, study design, eligibility criteria, participant baseline characteristics, treatment regimen, sampling method(s), prognostic factor with measurement and assessment methods, outcome data and measurement, follow-up duration, analysis details including adjustment (potential confounding) factors, association statistics with measures of variance, losses to follow-up. We extracted adjusted and, where appropriate, unadjusted measures of association and planned to convert effect sizes as necessary. Confounding factors were also considered as part of the risk of bias assessment.

Risks of bias were independently assessed by two review authors using the Quality in Prognosis Studies (QUIPS) tool. Any disagreements were resolved through discussion and consensus. The QUIPS tool considers the following six domains: representative population, missing data, prognostic factor measurement, outcome measurement, confounding factors, analysis and reporting. We assigned overall risks of bias as low, moderate or high based on predetermined criteria across all assessed domains. Associations were considered to have a low risk overall risk of bias where all domains were low risk or where one was moderate but the rest were low; where two or more domains were at moderate risk of bias and none at high risk the overall risk of bias was considered to be moderate; where one or more domains was at high risk of bias the overall risk of bias was considered to be high. For each study we summarised the results of the risk of bias assessment for each domain, and overall, in a table (see Supporting Information Tables); a full assessment which includes the comments for each study for each domain is available on request from the authors.

We used a modified GRADE framework to assess the quality of evidence for each prognostic factor–outcome combination.
assessments were carried out by one review author and checked by a second; disagreements were resolved through discussion and consensus. GRADE takes into account the results of the risk of bias assessment of contributing studies, but also whether there is imprecision, inconsistency, indirectness or publication bias in the evidence for each association. Evidence may be graded as high, moderate, low or very low certainty based on the assessment. In some cases we adopted a flexible approach where areas of concern across multiple domains were aggregated to produce a single downgrading; this approach is supported by the GRADE working group. GRADE judgements for each association, their rationale and the evidence on which they are based are summarised in the Summary of Findings Tables (see Supporting Information). Where evidence varied in completeness, we gave precedence to the results of cohorts with fully reported adjusted effect estimates for the association in determining the GRADE assessment; whilst considering the congruence of this data with that from studies with incomplete reporting. Where we needed to select an effect estimate for the Summary of Findings Table we gave priority to cohorts with low or moderate risk of bias over those with high risk of bias, while acknowledging the results of higher risk cohorts.

2.1.4 | Data synthesis

We conducted a narrative synthesis supported by structured tables, with evidence from studies grouped, as planned in the protocol, by the prognostic factor reported, the baseline infection status and the wound aetiology, because of the clinical relevance of these factors and the likely different treatment regimens in practice. We had planned to implement a random-effects meta-analytic approach where appropriate; however, due to the clinical and methodological heterogeneity of the prognostic variables and the often limited reporting of the data this was not possible. Where appropriate we have presented studies using forest plots but have not calculated pooled association statistics. Because of the disparate and sparse nature of the data we have also mapped the evidence and the gaps in the evidence graphically. In the body of the paper we have prioritised presenting detailed evidence summaries in the text for potential prognostic factors with most clinical salience (e.g., infection and presence of organisms with resistant characteristics) and those for which the evidence may be more generally applicable in a clinical setting (broader groupings such as anaerobic bacteria, pathogenic bacteria or Gram-negative or -positive bacteria). We have fully documented the evidence for all individual species in the Supporting Information and have provided a summary of those for which there was low certainty evidence (as opposed to very low certainty evidence) reported by more than one cohort.

3 | RESULTS

We identified 28 cohorts from 21 studies (in 23 publications) for inclusion in the review; together these report data on 38,604 participants. Some studies reported on more than one cohort (cohorts with different wound aetiologies or RCT treatment groups analysed as cohorts). These studies were identified from screening of 913 records following deduplication of the searches; we identified a further six studies from reference checking and assessed 272 studies as full text. In addition to the 21 included studies, there were 22 studies which would otherwise have met the inclusion criteria but did not contain any multivariable analysis or use any other appropriate methods (see Appendix). The review process is documented in the PRISMA diagram (Figure 1).

3.1 | Characteristics of cohorts

The characteristics of the included cohorts are summarised in Figure 2. Figure 2(A) summarises characteristics by wound aetiology and Figure 2(B) summarises the same cohorts but by baseline infection status. Detailed information on the characteristics of the cohorts is shown in Table S1.
Of the 28 cohorts, 15 had a prospective cohort study design or were RCTs analysed as cohort studies, and the remainder were retrospective cohorts, or drawn from retrospectively analysed registry data. Of the 38,604 participants who contributed data to this review 90% (34,675) were from six cohorts using registry data; analyses of these data were reported in three overlapping studies.4,6,35 Most other studies were small (total participants 3929; median 136; range 64–1340).

Twenty-two of the 28 cohorts included participants whose wounds had mixed infection status at baseline (some participants had infected wounds at baseline and some had non-infected wounds).4,6,31-43 This represents the overwhelming majority of participants (Figure 2(B)). Most cohorts of participants with mixed infection status wounds assessed the role of clinical infection (variably defined) as a prognostic factor for healing (Figure 3). While all four wound aetiologies were well represented in terms of total participant numbers they varied in the source of data and only registry data (for which reporting was limited) were available for pressure ulcers (Figure 2(A)).

In six smaller cohorts, of people with foot ulcers in diabetes or venous leg ulcers, infection was used as an inclusion or exclusion criterion and the studies assessed a wide range of relevant bacteria-related prognostic factors. Four cohorts (446 people) included only people with wounds which were not infected at baseline.13,17,27,28 Two cohorts (both in people with diabetes and foot ulcers; 487 participants) enrolled only people with wounds which were infected at baseline.29,30

There was considerable variation in how healing was reported in cohorts including the time to complete healing, the proportion of wounds completely healed at a given follow-up and change in wound area (a secondary review outcome and not analysed where primary outcome was reported). Follow-up duration varied considerably, ranging from 10 weeks to 1 year. The adjusting variables taken into consideration in the multivariable analyses also varied in number and type; most studies used some demographic and some wound characteristics; age and wound size at baseline were the most common. Several studies looked at a large number of potential factors in initial analyses and employed prespecified rules on significance for those used in subsequent multivariable analyses. The consideration of appropriate confounding factors formed part of our assessment of risk of bias for each association. Details are given in Tables S2–S5.

3.2 | Quality of the evidence

We assessed the overall quality of the evidence using the GRADE approach for prognostic factor evidence.25 Risk of bias was one element considered in the quality assessment and this varied; nine cohorts were considered to produce findings at moderate risk of bias but two cohorts produced findings judged at low risk,40,42 and 17 cohorts (from 10 reports) produced findings judged at high risk of bias.4,6,13,27,28,33,35,36,39,43 The great majority of participants were in the majority of cohorts judged to produce findings at high risk of bias. A summary of the QUIPS assessment is shown in Table S2. The domain which was most commonly judged to be at high risk of bias was analysis and reporting, whilst that most rarely judged to be at high risk of bias was prognostic factor measurement; high risk of bias was equally common across the other four domains. Inclusion of appropriate confounding factors is one of these domains and cohorts reported adjusting for a range of factors at the level of the participant and the wound. Age and gender were the most common person-level factors, while wound duration and measures of wound size were the most common wound level factors. Full details are given in Tables S3–S5.

As previously noted, evidence was also assessed for inconsistency, imprecision, indirectness and publication bias. Evidence for most of the prognostic factors evaluated was downgraded at least once for risk of bias; other downgrading decisions varied but almost all evidence was assessed as either low or very low certainty, with many associations downgraded at least once for imprecision. Publication bias was considered likely in evidence for the association between infection and wound healing.

3.3 | Association between baseline wound infection status and healing

Seventeen cohorts (36,472 participants) assessed the association between baseline wound infection (presence or absence of infection, defined in various ways) and wound healing, assessed as either proportion of wounds healed or time to complete healing.31-35,42 Data could not be pooled and are presented narratively and summarised in Figure 3; further details are in Table S3. Figure 3 also contains GRADE assessments for the evidence for each wound aetiology and the full assessments are summarised in Summary of Findings tables in Supporting Information.

Definitions of infection were not given in several instances. Other cohorts reported that presence of one or two key indicators was used to determine infection status. (Table S3). A minority of studies used established definitions of wound infection or supplied detailed criteria for this.

A large proportion of the data derived from cohorts with a high risk of bias, and a majority of participants were in cohorts with limited reporting of adjusted effect estimates for the association between infection and healing; several cohorts only reported P-values or other summary data which could not meaningfully be interpreted in the absence of additional data. Reported data are shown in Table S3. As outlined, we prioritised data from fully reported adjusted analyses, where these were available, in forming our GRADE assessments, while taking into account the agreement with evidence from other cohorts and their data.

Adjusted analyses with numerical results for associations were reported for four cohorts with 1229 participants. Of these 393 participants (one cohort) had open surgical wounds and 836 (three cohorts) had foot ulcers and diabetes (Figure 4). All four cohorts reported adjusted estimates showing an association between wound infection and reduced likelihood (risk) of healing.
|                  | A. Wound aetiology                                                                 | B. Baseline infection status                                                                 |
|------------------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| **Foot ulcers in** | **Mixed infection status**  13 cohorts 8049 people                                | **Foot ulcers in diabetes**  13 cohorts 8049 people                                        |
| diabetes         | Registry study 1 cohort 5794 people                                               | Registry study 1 cohort 5794 people                                                        |
|                  | **Other study type**  12 cohorts 2255 people                                      | **Other study type**  12 cohorts 2255 people                                                |
|                  | **Infected**  2 cohorts 487 people                                                 | **Venous leg ulcers**  3 cohorts 11970 people                                             |
|                  | Registry study                                                                    | Registry study 1 cohort 11773 people                                                       |
|                  | **Other data**  2 cohorts 487 people                                               | **Other study type**  2 cohorts 197 people                                                  |
|                  | **Uninfected**  1 cohort 84 people                                                 | **Pressure ulcers**  2 cohorts 10323 people                                               |
|                  | Registry study                                                                    | Registry study 2 cohorts 10323 people                                                       |
|                  | **Other study type**  1 cohort 84 people                                           | **Other study type**                                                                        |
| **16 cohorts**   | 8620 people                                                                        | **Mixed infection status wounds**  22 cohorts 37671 people                                 |
|                  |                                                                                   | **Foot ulcers in diabetes**  13 cohorts 8049 people                                        |
|                  |                                                                                   | Registry study 1 cohort 5794 people                                                        |
|                  | **Infected**  2 cohorts 487 people                                                 | **Other study type**  12 cohorts 2255 people                                                |
|                  | Registry study                                                                    | **Venous leg ulcers**  3 cohorts 11970 people                                             |
|                  | **Other data**  2 cohorts 487 people                                               | Registry study 1 cohort 11773 people                                                       |
|                  | **Uninfected**  1 cohort 84 people                                                 | **Other study type**  2 cohorts 197 people                                                  |
|                  | Registry study                                                                    | **Pressure ulcers**  2 cohorts 10323 people                                               |
|                  | **Other study type**  1 cohort 84 people                                           | Registry study 2 cohorts 10323 people                                                       |
|                  |                                                                                   | **Other study type**                                                                        |
| **Venous leg ulcers** | **Mixed infection status**  3 cohorts 11970 people                                | **Foot ulcers in diabetes**  2 cohorts 487 people                                        |
|                  | Registry study 1 cohort 11773 people                                               | Registry study 2 cohorts 487 people                                                        |
|                  | **Other study type**  2 cohorts 197 people                                         | **Other study type**                                                                        |
|                  | **Infected**                                                                      | **Venous leg ulcers**                                                                      |
|                  | Registry study                                                                    | Registry study                                                                          |
|                  | **Other study type**                                                               | **Other study type**                                                                        |
|                  | **Uninfected**  3 cohorts 362 people                                               | **Pressure ulcers**                                                                        |
|                  | Registry data                                                                     | Registry study                                                                           |
|                  | **Other study type**  3 cohorts 362 people                                         | **Other study type**                                                                        |
| **6 cohorts**    | 12332 people                                                                       | **Open surgical wounds**  4 cohorts 7329 people                                           |
|                  |                                                                                   | Registry study 2 cohorts 6785 people                                                       |
|                  | **Infected**                                                                      | **Other study type**                                                                        |
|                  | Registry study                                                                    | **Pressure ulcers**                                                                        |
|                  | **Other study type**                                                               | Registry study                                                                           |
|                  | **Uninfected**  3 cohorts 362 people                                               | **Other study type**                                                                        |
|                  | Registry study                                                                    | **Open surgical wounds**                                                                  |
|                  | **Other study type**                                                               | Registry study 1 cohort 84 people                                                         |
| **Pressure ulcers** | **Mixed infection status**  2 cohorts 10323 people                                | **Foot ulcers in diabetes**  1 cohort 84 people                                           |
|                  | Registry study 2 cohorts 10323 people                                              | Registry study                                                                           |
|                  | **Other study type**                                                               | **Other study type**                                                                        |
|                  | **Infected**                                                                      | **Venous leg ulcers**                                                                      |
|                  | Registry study                                                                    | Registry study                                                                           |
|                  | **Other study type**                                                               | **Other study type**                                                                        |
|                  | **Uninfected**  3 cohorts 362 people                                               | **Pressure ulcers**                                                                        |
|                  | Registry study                                                                    | Registry study                                                                           |
|                  | **Other study type**                                                               | **Other study type**                                                                        |
| **2 cohorts**    | 10323 people                                                                       | **Open surgical wounds**  4 cohorts 7329 people                                           |
|                  |                                                                                   | Registry study                                                                           |
|                  | **Uninfected**  3 cohorts 362 people                                               | **Other study type**                                                                        |
|                  | Registry study                                                                    | **Pressure ulcers**                                                                        |
|                  | **Other study type**                                                               | Registry study                                                                           |
|                  |                                                                                   | **Other study type**                                                                        |
| **Open surgical wounds** | **Mixed infection status**  4 cohorts 7329 people                                | **Foot ulcers in diabetes**  1 cohort 84 people                                           |
|                  | Registry study 2 cohorts 6785 people                                              | Registry study                                                                           |
|                  | **Other study type**  2 cohorts 544 people                                         | **Other study type**                                                                        |
|                  | **Infected**                                                                      | **Venous leg ulcers**                                                                      |
|                  | Registry study                                                                    | Registry study                                                                           |
|                  | **Other study type**                                                               | **Other study type**                                                                        |
|                  | **Uninfected**  3 cohorts 362 people                                               | **Pressure ulcers**                                                                        |
|                  | Registry study                                                                    | Registry study                                                                           |
|                  | **Other study type**                                                               | **Other study type**                                                                        |
| **4 cohorts**    | 7329 people                                                                       | **Open surgical wounds**                                                                  |
|                  |                                                                                   | Registry study                                                                           |
|                  | **Uninfected**                                                                      | **Other study type**                                                                        |

**FIGURE 2** Map of evidence distribution by (A) Wound etiology and (B) Baseline infection status.
The cohort in open surgical wounds was considered to be at low risk of bias (adjusted HR for likelihood (risk) of healing if wound infected 0.65; 95% CI 0.51 to 0.83), and this was affected by indirectness as not all infections were present at baseline measurement; it was unclear if infection timing was considered in the analysis. This is moderate certainty evidence (downgraded once for indirectness) that wound infection in an open surgical wound probably reduces the likelihood of healing.

One of the cohorts in people with foot ulcers was at moderate risk of bias (118 participants; adjusted HR 0.53; 95% CI 0.33 to 0.83). The other two cohorts (204 participants) were at high risk of bias and we were very uncertain about the reliability of the reported analyses. This is low certainty evidence which was downgraded twice for high risk of bias impacting most of the participants.

Four cohorts of people with foot ulcers in diabetes (342 participants) reported unadjusted association measures or data to allow calculation of these. These results were not consistent with the studies which reported adjusted effect measures; all except one cohort had confidence intervals which included the possibility of benefit as well as harm to infected status. It is possible that, in addition to imprecision from low numbers of participants/events, the role of treatments in the cohorts drawn from an RCT may have been a factor in this.

Studies which reported only P values almost all reported statistically significant values for the association between infection and complete wound healing.
healing. This included the very large registry cohorts. Whilst these data are subject to very high risks of bias, they represent large numbers of participants and show effects which align with those from the fully reported multivariable analyses. In addition to reporting issues in the cohorts which reported only p values we consider that there is potential for publication bias, with small studies which did not find clear evidence for an association between infection and healing possibly missing from the published literature. This should be taken into consideration when assessing the certainty of the evidence.

In summary, there is low certainty evidence for an association between wound infection at study baseline and subsequent poorer healing (defined as a longer time to heal or a lower probability of healing) for foot ulcers in people with diabetes and some moderate certainty evidence for this association in open surgical wounds, based on those studies with a fully reported adjusted effect size (Figure 3), and taking other studies into consideration. Evidence for the association between infection and healing in pressure ulcers or venous leg ulcers is uncertain (very low certainty) because of the combined impact of high risks of bias, imprecision and potential publication bias. We consider the evidence for an association between wound infection and a reduced likelihood (risk) of healing across all wound types to be low certainty, taking into account all the cohorts which contributed evidence. The evidence and the GRADE assessments for certainty are summarised in the Summary of Findings Table (Supporting Information).

3.4 | Other bacterial prognostic factors for wound healing

A wide range of other potential prognostic factors were assessed, primarily in studies which enrolled only people with infected foot ulcers in diabetes or only people with uninfected venous leg ulcers or foot ulcers at baseline. These potentially prognostic factors can be broadly summarised as bacterial load; bacterial diversity; the presence of specific types of bacteria either at the level of broad characteristics (e.g., pathogens; anaerobic; Gram-positive; Gram-negative) or the phylum, family, genera, species or subspecies. Many of these data were limited by being at high risk of bias as well as imprecision.

We have mapped the prognostic evidence for the listed microbiome factors and wound healing risk by wound type and wound infection status (Figure 5), data for family, genera, species or subspecies is presented narratively. None of the potential prognostic factors assessed showed a clear association with the likelihood (risk) of healing. As Figure 5 demonstrates, the evidence available on which to base inference is limited: no data are available for pressure ulcers and very little is reported for open surgical wounds. There is more information for associations of these microbiome wound factors with healing in venous leg ulcers and foot ulcers in people with diabetes, but most associations are represented by a single cohort. Risk of bias varied from low to high, but almost all associations were affected by substantial imprecision, leading to the evidence being downgraded twice which means that evidence was low certainty and, where there was also high risk of bias, very low certainty. Details of the adjusted analyses for each potential prognostic factor are given in the Appendix and Tables S4 and S5. The evidence and the GRADE assessments for certainty are summarised in the Summary of Findings Table (Supporting Information).

3.5 | Individual families and species of microorganisms

Studies assessing individual families or genera of microorganisms looked at both infected and uninfected wounds and included foot ulcers in people with diabetes, venous leg ulcers and post-amputation open surgical wounds. Adjusted estimates were reported only for foot ulcers in diabetes. Most evidence was low or very low certainty (downgraded for imprecision in each case, and in some cases also for high risk of overall bias). Full details are given in Table S5 and the
Supporting Information; we discuss evidence where there were adjusted effect estimates and low certainty evidence. The great majority of the species assessed as potential prognostic factors did not show a clear association with healing in any type of wound. The exception was coagulase-negative *Staphylococcus*, where a cohort of 299 people with baseline infection of foot ulcers in diabetes found low certainty evidence that there may be an association with longer time to complete healing (adjusted HR for healing 1.53; 95% CI 0.98 to 2.40).\textsuperscript{29} This was low certainty evidence downgraded twice for imprecision.

The most commonly assessed species was *Staphylococcus aureus* where the association with healing was assessed in six cohorts (801 participants), followed by strains of *Streptococcus* (five cohorts, 731 participants)\textsuperscript{17,29,39-41}; and *Pseudomonas* (five cohorts, 711 participants).\textsuperscript{27,29,39-41} Adjusted effect estimates were available for *S. aureus* for two cohorts (393 participants)\textsuperscript{17,29}; for *Streptococcus* these two cohorts also reported adjusted effects,\textsuperscript{17,29} while for *Pseudomonas* only a single cohort (299 participants) did so.\textsuperscript{29} In each case adjusted effect estimates were available only for foot ulcers and there was low certainty evidence of no clear association of the bacteria with the likelihood (risk) of healing. The certainty of the evidence was downgraded twice for imprecision. Other species or groups were assessed only by single cohorts.

### DISCUSSION

#### 4.1 Summary of the evidence

To the best of our knowledge this is the first systematic review of microbiological potential prognostic factors for complex wound healing. We developed our protocol prior to the publication of the

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**FIGURE 5** Map of evidence for characteristics of wound microbiome as potential prognostic factors for wound healing [Color figure can be viewed at wileyonlinelibrary.com]
SWIM guidelines for synthesis without meta-analysis,45 however our approach follows many of the same principles of transparency. We considered a wide range of factors, from a diagnosis of clinical wound infection to the presence of individual species.

We identified 21 studies reporting on 28 cohorts; most were prospective studies but a minority including six cohorts of registry data were retrospective. GRADE assessments judged almost all the evidence to be low or very low certainty, largely due to high all-domain risks of bias and imprecision.

There was some moderate certainty evidence that a diagnosis of wound infection may be prognostic of poorer healing in open surgical wounds and low certainty evidence in foot ulcers in diabetes. Across wound etiologies the evidence is low certainty. Low certainty evidence means that more research could change these findings. Findings for almost all other bacterial prognostic factors assessed showed no clear relationship with wound healing, or too much uncertainty to determine if there was a relationship.

4.1.1 | Mixed wound infection status studies

Cohorts that recruited people with wounds that could be either infected or noninfected at baseline generated the majority of participants in this review. Infection is consistently associated with a lower likelihood of complete healing/longer time to complete healing in multivariable analyses in foot ulcers in people with diabetes and in open surgical wounds. However, effect sizes and definitions of infection varied or were absent, and the certainty of the evidence is reduced by the high risks of bias in many studies, by incomplete reporting, and by indirectness. Publication bias is considered likely in prognosis research and is probably an additional limitation for this association across wound types.44 Another limitation was the fact that several studies did not report how they defined wound infection in their baseline assessment (see Tables S2 and S3). The evidence for wound infection being prognostic for wound healing had very low certainty in pressure ulcers and venous leg ulcers.

In determining the certainty of the evidence we considered carefully whether observed association sizes should prompt uprating of the evidence for moderate or large effect size, but the studies which might have given rise to this decision were at high risk of bias in more than one domain and the risk of bias might have led to inflated association statistics sizes. Evidence from these mixed infection status cohorts for other potential prognostic factors was very limited.

4.1.2 | Wounds infected at baseline

Where wounds are infected at baseline, studies evaluated several potential microbiological prognostic factors in foot ulcers in diabetes including the presence of multiple individual genera or species of bacteria but, in most cases, the there is no clear association with healing. This is low certainty evidence because of imprecision.

There is no evidence for any other type of wound where there is baseline infection.

4.1.3 | Wounds without baseline infection

In wounds without an infection at baseline, studies also evaluated several potential microbiome-related prognostic factors in foot ulcers in diabetes, including bacterial load and diversity as well as individual genera or species but in no case did any study find a clear association with healing. There is very limited and uncertain evidence in venous leg ulcers without baseline infection. There were no studies assessing potential prognostic factors in any other type of wound where there is no baseline infection.

4.2 | Relationship of the evidence base to clinical practice

There are many potential microbiological prognostic factors for wound healing in complex wounds. The studies we identified collectively assessed a large number of these. In almost all cases there was no clear association, or too much uncertainty to determine if there was an association, between the potential factor and wound healing. The exception is evidence that clinical infection is associated with poorer healing for foot ulcers in diabetes (low certainty) and open surgical wounds (moderate certainty) but even this may be affected by publication bias. Even though this is low certainty evidence, considered across all wound types, the taking of steps to prevent wound infection remains clinically important for a range of reasons. Treating infections clearly also remains important.

Whilst wounds that are infected may be at increased risk of poor healing, this association requires further exploration and findings of this work might guide future development of treatment regimes. It is important to note that an association between a prognostic variable and an outcome does not imply that there is a causal relationship.46 Clinical wound infection may be reflective of a sub-optimal wound status at baseline rather than a cause of it. Although the included studies adjusted for some factors in their analyses it is highly likely that underlying issues at the level of the wound or person played a role in determining both susceptibility to infection and healing trajectory in the wounds evaluated. We know that there is limited evidence for the effect of antimicrobial treatments on healing in the types of complex wounds included here.22,47 We did not identify any studies that looked at the relationship between microbiological responses to treatment and wound healing, although antibacterial treatments were used and adjusted for in analyses in studies in infected wounds.

It has been proposed that other features of wound microbiomes (bacterial load, diversity and presence of particular types of bacteria or resistant strains of bacteria) may be predictive of or responsible for poor healing of complex wounds.19 The evidence we identified was relatively limited and what data were available on potential factors assessed showed no clear relationship with wound healing.
particular, we did not find evidence to support a prognostic relationship of nonclinical wound features such as the concept of “critical colonisation” with wound healing. Bacterial load and bacterial diversity were assessed only in wounds without baseline infection and, in each case, there was limited evidence which did not show clear associations between load.3,17 We identified only one study which specifically looked at colonisation,39 and one which looked at presence of biofilm.34 In both cases there were issues with the way in which these potential prognostic factors were assessed. There were no data which addresses whether “critical colonisation” may be associated with poorer healing, and the data on bacterial load were imprecise, being drawn from small cohorts.

This summary of the evidence shows that we do not know with any certainty whether the load or variety of bacteria, or the presence of particular groups of bacteria in complex wounds is independently associated with the healing of those wounds. Low numbers of participants contributed to substantial imprecision around most measures of association, and in many cases the data came from studies at high risk of bias. Our findings are in line with guidance which suggests restricting use of many antimicrobial dressings to wounds with clinical infection.2

There is a particularly clear gap in the evidence for possible microbiological prognostic factors for the healing of pressure ulcers, especially given that the healing trajectory for these wounds can be slow and difficult to predict once mechanical factors are controlled for. We have limited evidence for the effect of factors other than clinical infection on healing in other complex wounds. There is also a clear need for more-well-conducted and reported studies of potential microbiological prognostic factors in complex wounds including foot ulcers. Longitudinal studies which assess both the response of the wound microbiome to antimicrobial treatment and the time to wound healing would help to inform our understanding of the relationship between bacterial factors and healing processes. While these studies would improve our understanding of what may predict poor wound healing it is important to remember that they would not address the question of whether there is a causal relationship of aspects of microbiology with healing; as with infection, other microbiological features may also reflect underlying factors such as reduced immunological response or poor perfusion, which also lead to reduced healing.48

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CONFLICT OF INTEREST
None of the authors have any conflicts of interest to declare.

DATA AVAILABILITY STATEMENT
This is a systematic review based entirely on previously published data. A great deal of the review process is documented in the supplementary information; further data from the review process such as lists of excluded studies or full details of the risk of bias assessment are available on request from the authors, as stated in the text.

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REFERENCES
1. Cullum N, Buckley H, Dumville J, Hall J, Lamb K, Madden M. Wounds research for patient benefit: a 5-year programme of research. Prog Grants Appl Res. 2016;4(13):1-303.
2. (NICE) NIfHaCE. Chronic wounds: advanced wound dressings and antimicrobial dressings. March 2016. https://www.nice.org.uk/advice/esmpb2/chapter/Full-evidence-summary-medicines-and-prescribing-briefing; 2016
3. Hall J, Buckley H, Lamb K, Stubbs N, Saramago P, Dumville J. Point prevalence of complex wounds in a defined United Kingdom population. Wound Rep Reg. 2014;22(6):694-700.
4. Horn SD. Development of a wound healing index for patients with chronic wounds. Wound Rep Reg. 2013;21:823-832.
5. Margolis D, Allen-Taylor L, Hoffstad O, Berlin J. The accuracy of venous leg ulcer prognostic models in a wound care system. Wound Rep Reg. 2004;12(2):163-168.
6. Horn SDA. Predictive model for pressure ulcer outcome: the wound healing index. Adv Skin Wound Care. 2015;28(12):561-572.
7. Gardner S, Hillis S, Heilmann K, Segre J, Grice E. The neuropathic diabetic foot ulcer microbiome is associated with clinical factors. Diabet. 2013;62:923-930.
8. Sotto A. Virulence potential of Staphylococcus aureus strains isolated from diabetic foot ulcers: A new paradigm. Diabet Care. 2008;31(12):2318-2324.
9. Loeschke M, Gardner SE, Kalan L, et al. Temporal stability in chronic wound microbiota is associated with poor healing. J Invest Dermatol. 2017;137(1):237-244.
10. Malone M, Bjarnsholt T, McBain A, et al. The prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data. J Wound Care. 2017;26:20-25.
11. Omar A, Wright J, Schultz G, Burrell R, Nadworny P. Microbial biofilms and chronic wounds. Microorganisms. 2017;5(1):9.
12. Gilbert P, Maira-Litran T, McBain A, Rickard A, Whyte F. The physiology and collective recalcitrance of microbial biofilm communities. Adv Microb Physiol. 2002;46:202-256.
13. Davies CE. A prospective study of the microbiology of chronic venous leg ulcers to re-evaluate the clinical predictive value of tissue biopsies and swabs. Wound Rep Reg. 2008;15:17-22.
14. Trengove N, Stacey M, McGechie D, Stingemore N, Mata S. Qualitative bacteriology and leg ulcer healing. J Wound Care. 1996;5:277-280.
15. Bowler P. The 105 bacterial growth guideline: reassessing its clinical relevance in wound healing. Ostomy Wound Manag. 2003;49(1):44-53.
16. Dalton T, Dowd S, Wolcott R, et al. An in vivo polymicrobial wound infection model to study interspecies interactions. PLoS ONE. 2011;6:e27317.
17. Gardner SE, Haleem A, Joo YL, et al. Cures of diabetic foot ulcers without clinical signs of infection do not predict outcomes. Diabet Care. 2014;37(10):2693-2701.
18. Lebeaux D, Chauhan A, Rendueles O, Beloin C. From in vitro to in vivo models of bacterial biofilm-related infections. Pathogens. 2013;2:288-356.
19. Kingsley A, White R, Gray D. The wound infection continuum: a revised perspective. APW Suppl Wounds UK. 2004;1(1):13.
20. (AWMA) AWMA. Bacterial impact on wound healing: from contamination to infection. Version 1.5. October 2011. www.awma.com.au/publications/2011_bacterial_impact_position_1.5.pdf; 2011.
21. Michaels J, Campbell B, King B, Palfreyman S, Shackley P, Stevenson M. Randomized controlled trial and cost-effectiveness analysis of silver-donating antimicrobial dressings for venous leg ulcers (VULCAN trial). Br J Surg. 2009;96(10):1147-1156.
22. Norman G, Dumville J, Mohapatra D, Owens G, Crosbie E. Antibiotics and antisepsis for surgical wounds healing by secondary intention. Cochrane Database Syst Rev. 2016;3. https://doi.org/10.1002/14651858.CD011712.pub2.
23. Norman G. Bacteria and bioburden in complex wounds: a prognostic review. PROSPERO International prospective register of systematic reviews; 2019. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=136141.
24. Hayden J, Van der Windt D, Cartwright J, Côté P, Bombardier C. Assessing bias in studies of prognostic factors. Ann Intern Med. 2013;158(4):280-286.
25. Iorio A, Spencer F, Falavigna M, et al. Use of GRADE for assessment of evidence about prognosis: rating confidence in estimates of event rates in broad categories of patients. BMJ. 2015;350:h870. https://doi.org/10.1136/bmj.h870.
26. Schunemann H, Brozek J, Guyatt G, Oxman A. Introduction to GRADE Handbook. Handbook for Grading the Quality of Evidence and the Strength of Recommendations Using the GRADE Approach. https://gdt.gradepro.org/app/handbook/handbook.html; GRADE Working Group; 2013.
27. Hansson C, Hobom J, Moller A, Swanbeck G. The microbial flora in venous leg ulcers without clinical signs of infection. Repeated culture using a validated standardised microbiological technique. Acta Derm Venereol. 1995;75(1):24-30.
28. Lantis UC, Marston WA, Farber A, et al. The influence of patient and wound variables on healing of venous leg ulcers in a randomized controlled trial of growth-arrested allogeneic keratinocytes and fibroblasts. J Vasc Surg. 2013;58(2):433-439.
29. Ndosi M, Wright-Hughes A, Brown S, et al. Prognosis of the infected diabetic foot ulcer: a 12-month prospective observational study. Diabet Med. 2018;35(1):78-88.
30. Richard JL, Sotto A, Jourdan N, et al. Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers. Diabetes Metab. 2008;34(4 Pt 1):363-369.
31. Coepeer S, Schaffer M, Witte M, et al. Impact of local surgery on the healing of refractory diabetic foot ulcerations. Foot Ankle Surg. 2001;7(2):103-108.
32. Das. Predictors of delayed wound healing after successful isolated below-the-knee endovascular intervention in patients with ischemic foot ulcers. J Vasc Surg. 2018;67(4):1181-1190.
33. Edmonds M. Multicenter, randomized controlled, observer-blinded study of a nitric oxide generating treatment in foot ulcers of patients with diabetes-ProNOx1 study. Wound Rep Reg. 2018;26:228-237.
34. Formosa C. Characteristics predicting foot ulcer outcomes in the diabetic foot. Diabetic Complications [Internet]. SM E-Book; 2016. www.smgebooks.com.
35. Fife CE. A predictive model for diabetic foot ulcer outcome: The wound healing index. Adv Wound Care. 2016;5(7):279-287.
36. Ince. Use of the SINBAD classification system and score in comparing outcome of foot ulcer management on three continents. Diabetes Care. 2008;31(5):964-967.
37. Rhou YJJ, Henshaw FR, McGill MJ, Twigg SM. Congestive heart failure presence predicts delayed healing of foot ulcers in diabetes: an audit from a multidisciplinary high-risk foot clinic. J Diabetes Complications. 2015;29(4):556-562.
38. Wang A, Sun X, Wang W, Jiang K. A study of prognostic factors in Chinese patients with diabetic foot ulcers. Diab Foot Ank. 2014;5:22936.
39. Halbert AR, Stacey MC, Rohr JB, Jopp-McKay A. The effect of bacterial colonization on venous ulcer healing. Australas J Dermatol. 1992;33(2):75-80.
40. Moffatt CJ, Doherty DC, Smithdale R, Franks PJ. Clinical predictors of leg ulcer healing. Br J Dermatol. 2010;162(1):51-58.
41. Wong KL, Nather A, Liang S, Chang Z, Wong TTC, Lim CT. Clinical outcomes of below knee amputations in diabetic foot patients. Annals Acad Med Singapore. 2013;42(8):388-394.
42. Chetter I, Oswald A, McGinnis E, et al. Patients with surgical wounds healing by secondary intention: A prospective, cohort study. Int J Nurs Stud. 2019;69:62-71.
43. Spanos K, Athanasoulas A, Karanthos C, Bargiota A, Chan P, Giannounas A. Factors associated with ulcer healing and quality of life in patients with diabetic foot ulcer. Angiology. 2017;68(3):242-250.
44. Kyzas P, Denaxa-Kyza D, Ioannidis J. Almost all articles on cancer prognostic markers report statistically significant results. Eur J Cancer. 2007;43(17):2559-2579.
45. Campbell M, JE M, A S, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. BMJ. 2020;368:l6890.
46. Riley R, Hayden J, Steyerberg E, et al. Prognosis research strategy (PROGRESS) 2: prognostic factor research. PLoS Med. 2013;10(2); e1001380.
47. Dumville J, Lipsky BA, Hoey C, Cruciani M, Fiscon M, Xia J. Topical antimicrobial agents for treating foot ulcers in people with diabetes. Cochrane Database Syst Rev. 2017;6:CD011038. https://doi.org/10.1002/14651858.CD011038.pub2.
48. Ahmad A, Antonsen E. Immune and vascular dysfunction in diabetic wound healing. J Wound Care. 2016;25(Suppl 7):S35-S46.

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

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