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Bacterial Bioprospecting for Obtaining Antibiotic Substances against *Staphylococcus aureus* and *Pseudomonas aeruginosa*: A Literature Review

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Abstract

The decline in antibiotic development, alongside the increasing prevalence of multidrug-resistant bacteria, highlights the urgent need for new antimicrobial agents. Among the pathogens prioritized by the World Health Organization for antimicrobial research and development are *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are associated with severe infections and significant resistance mechanisms that compromise treatment efficacy. In this context, bioprospecting has emerged as a promising strategy for identifying new antimicrobial compounds from diverse biological sources. This integrative literature review synthesized current methodologies used for the isolation, cultivation, processing, extraction, and identification of antibiotic-producing bacteria active against *S. aureus* and *P. aeruginosa*, providing an overview of approaches applied to pathogens of critical clinical relevance. The review was conducted using the MEDLINE portal through the PubMed database, and included *in vitro* studies published in English between 2020 and 2025 that aligned with the proposed objective, resulting in 44 eligible articles. The findings demonstrated that marine environments, soil, food sources, and the human microbiota are the primary sources of bacteria with antimicrobial potential against these pathogens. Lactic acid bacteria were the most frequently

identified group, although other bacterial genera were also reported, such as *Bacillus*. Cultivation conditions varied according to the bacterial species and study objectives. Antibiotic substances were predominantly obtained through solvent extraction and ammonium sulfate precipitation, while structural characterization relied mainly on chromatographic techniques, nuclear magnetic resonance spectroscopy, and mass spectrometry. Overall, this review consolidates current methodological strategies in antimicrobial bioprospecting and may support future studies focused on the discovery and development of new antibacterial compounds targeting WHO priority pathogens.

Keywords

Antibacterial Agents, Bioprospecting, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Chromatography

1. Introduction

The decline in antibiotic development [1] has occurred in parallel with the emergence of multidrug-resistant bacteria, that is, those that are resistant to more than three classes of antibiotics [2] [3]. Therefore, the search for new antimicrobial drugs is of great importance, given that some classic pathogens have a great capacity to acquire and disseminate resistance genes, making them global public health problems [4]-[7].

In 2017, the World Health Organization (WHO) published a list of pathogens for which the development of new antimicrobials is an urgent matter, with the aim of guiding research and development of these drugs. In this context, the ESKAPE group, an acronym for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* sp., was designated as a priority target [8] [9].

Staphylococcus aureus is a Gram-positive bacterium and one of the most widespread pathogenic species, capable of causing serious infections such as respiratory tract infections, surgical site infections, cardiovascular infections, nosocomial infections, and skin infections, since this pathogen is commonly found on the skin of healthy people, being able to invade mucous membranes or penetrate the skin barrier in cases of wounds [10]-[13]. *S. aureus* bacteremia has an incidence rate between 20 and 50 cases per 100,000 people per year, with a mortality rate of 10 to 30% [13] [14]. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are resistant to several β-lactams commonly used in clinical practice, such as methicillin itself, penicillin, amoxicillin, and oxacillin [15]. Methicillin resistance rates in clinical isolates can reach more than 50% of cases in the United States and China [16], 25.9% for healthcare-associated bloodstream infections in Brazil [17] and are on the rise in developing countries in Africa [13] [18].

Pseudomonas aeruginosa is a motile, non-fermenting Gram-negative bacterium commonly found in aquatic environments such as lakes, rivers, and swim-

ming pools, as well as on plants, fruits, soil, animals, food, and in humans [19]-[22]. It is capable of causing opportunistic infections, especially in immunocompromised patients, post-surgical patients, or those in intensive care units, as well as patients with cystic fibrosis, burns, or diabetes mellitus [22]-[27]. It is also the most frequent colonizer of medical devices and one of the pathogens most associated with nosocomial infections [20] [28]. This bacterium commonly exhibits multidrug resistance characteristics, both naturally occurring and acquired, which makes the treatment of infections caused by this pathogen a challenge [20] [29].

The increasing prevalence of multidrug-resistant pathogens underscores the urgency of exploring safer alternative treatments [30] [31]. Conventional approaches, such as the chemical modification of existing antibiotics and synthetic drug development, have faced limitations due to high costs, long development timelines, and the rapid emergence of bacterial resistance. In this scenario, a possible alternative would be bioprospecting, which is characterized as the exploration of biodiversity in search of new biological resources with economic and social value. This process is carried out by various industries, notably the pharmaceutical industry, but also in sectors such as agriculture, manufacturing, engineering, construction, and others [32] [33].

This study aimed to analyze the methodological approaches used for the isolation, cultivation, and processing of antibiotic producing bacteria active against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, focusing on their sources, diversity, growth conditions, and the main methods employed for the extraction and identification of bioactive molecules.

2. Materials and Methods

2.1. Type of Study and Field of Research

This is an integrative literature review, conducted using the MEDLINE portal and the PubMed database. The PICo strategy (acronym for Population/Problem, Interest, and Context) was used to formulate the research question, enabling the identification of descriptors that aid in locating relevant primary studies in the databases [34]. **Table 1** illustrates the formulation of the research question using this strategy.

Table 1. Formulating the research question—PICo strategy.

PICo strategy	
P	Bacteria that produce antibiotic substances against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
I	Strategies for obtaining, cultivating and processing, including growing conditions and methodologies for extraction and identification.
Co	Characterization of the sources of isolation, diversity, conditions and methods used.

Thus, the delimited research question is: "What are the main strategies for ob-

taining, cultivating, and processing bacteria that produce antibiotic substances against *Staphylococcus aureus* and *Pseudomonas aeruginosa* described in the literature, considering their sources of isolation, taxonomic diversity, growth conditions, and methods of extraction and identification of bioactive molecules?" The keywords were selected from the Health Sciences Descriptors of the BVS and MeSH Database, using Boolean operators "AND" and "OR" to combine the terms: Anti-Bacterial Agents; Peptides; Bacteriocins; Lipopeptides; Enzymes; Biofilms; Bioprospecting; Metabolome; Microbiota; Supernatant; Wound healing; Wound infection; Cell-free supernatant; Cell-free extract; Chromatography, High Pressure Liquid; Liquid Chromatography-Mass Spectrometry; Nuclear Magnetic Resonance, Biomolecular; Proton Magnetic Resonance Spectroscopy; Magnetic Resonance Spectroscopy; *Staphylococcus aureus*; *Pseudomonas aeruginosa*.

2.2. Sampling

The sampling method used was sequential, which consists of recruiting all accessible productions that meet the eligibility criteria over a specific time interval [35], which was conveniently limited to the last five years.

The inclusion criteria used are: *in vitro* studies, adherence to the objective and the proposed theme, articles published in full, in English, with a publication time frame from 2020 to 2025.

Exclusion criteria: Duplicate articles, literature reviews, texts unavailable in full format, isolated case reports, opinion articles, and those that did not address the defined topic.

Data was collected from the PubMed database, maintained by the National Institutes of Health (NIH). This database was selected because of its broad coverage of biomedical and microbiological literature relevant to the scope of this review. The respective descriptors and Boolean operators were used: [(((Anti-Bacterial Agents) OR (Peptides) OR (Bacteriocins) OR (Lipopeptides) OR (Enzymes) OR (Biofilms)) AND ((Bioprospecting) OR (Metabolome) OR (Microbiota) OR (Supernatant) OR (Wound Healing) OR (Wound Infection) OR (cell-free supernatant) OR (cell-free extract) OR (Chromatography, High Pressure Liquid) OR (Liquid Chromatography-Mass Spectrometry) OR (Nuclear Magnetic Resonance, Biomolecular) OR (Proton Magnetic Resonance Spectroscopy) OR (Magnetic Resonance Spectroscopy)) AND ((*Staphylococcus aureus*) OR (*Pseudomonas aeruginosa*)) NOT (plants) NOT (herbal) NOT (Oils, Volatile) NOT (oils) NOT (phytochemicals) NOT (intestine) NOT (*in vivo*) NOT (mice) NOT (Capsules) NOT (gut) NOT (dental plaque) NOT (probiotics) NOT (seaweed) NOT (fungi) NOT (virus) NOT (silver) NOT (zinc) NOT (gold) NOT (metals) NOT (Hydrogels) NOT (Review [Publication Type]))]. Restrictive NOT filters related to probiotics and gut-associated terms were applied to narrow the scope of the review and avoid the overrepresentation of studies involving probiotic microorganisms and intestinal microbiota, which are already extensively described in the literature. The objective of these exclusions was to focus the search on less explored bacterial

sources with potential for antibacterial bioprospecting.

After searching the aforementioned database, the articles were refined beforehand by reading the abstract and selected after a complete reading of the text, to ensure they met the inclusion criteria. A flowchart of the search was created, containing the databases and the number of publications located. Search filters were also applied, based on the inclusion and exclusion criteria, to select potential bibliography for this study using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), as shown in the flowchart (**Figure 1**).

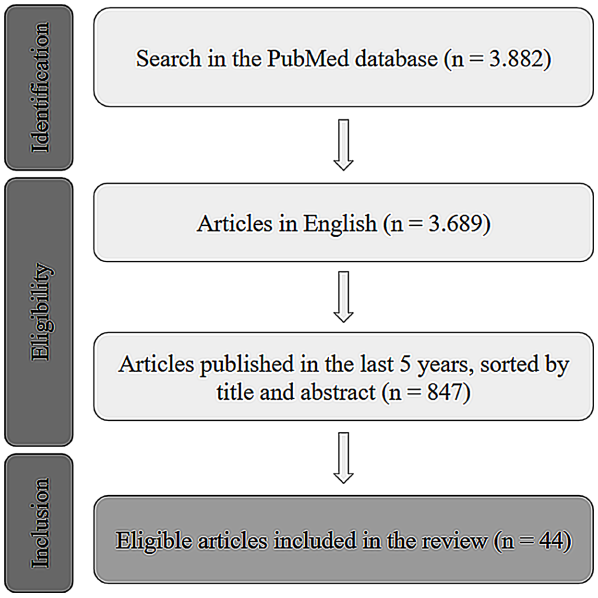

Figure 1. Article selection flowchart.

After applying the descriptors and Boolean operators in PubMed/MEDLINE, a total of 3882 articles were identified. Subsequently, filters for articles published in English and within the last five years were applied, resulting in 3689 and 847 articles, respectively. No duplicate articles were identified due to the use of a single database. The titles and abstracts of the 847 articles were screened, and 44 studies were considered eligible and included in the review after full-text assessment. The main reasons for exclusion during the screening process were studies conducted exclusively or predominantly *in vivo* (n = 79), articles not adherent to the proposed objective and theme of the review (n = 521), literature reviews (n = 194), case reports (n = 7), and opinion articles (n = 2). Study selection and data extraction were performed by a single reviewer according to the established eligibility criteria. Due to the integrative nature of this review and the methodological heterogeneity among the included *in vitro* studies, no formal methodological quality appraisal tool was applied. The studies were evaluated based on their relevance to the proposed objective and the established eligibility criteria.

The data extracted from the selected studies were organized into a spreadsheet, arranged in manually assigned sequential numbers, and which includes information such as number, authors, year, title, genera or species of antibiotic-pro-

ducing bacteria and their culture parameters, source of isolation of the producing bacteria, target species of the study, molecule extraction method used, identification method used, and nature or identification of the bioactive molecule.

The methods used to identify the biomolecules present in the supernatants were classified as follows:

- Separation techniques only: Thin Layer Chromatography (TLC), Cation Exchange Chromatography (CIEX), C18 chromatographic column, High-performance liquid chromatography (HPLC), Ion Exchange Chromatography (IEX), Reversed Phase High-performance liquid chromatography (RP-HPLC), Reversed-phase Chromatography (RPC) and Size Exclusion Chromatography (SEC);
- Spectroscopic techniques: Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR);
- Spectrometric techniques: Mass Spectrometry (MS), Electrospray Ionization Mass Spectrometry (ESI-MS) and Electrospray Ionization coupled to Mass Spectrometry (ESI-MS/MS);
- Electrophoretic techniques: Polyacrylamide Gel Electrophoresis (PAGE) and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE);
- Hybrid techniques: Gas Chromatography coupled to Mass Spectrometry (GC-MS), High Performance Liquid Chromatography coupled to Mass Spectrometry (HPLC-MS), High Performance Liquid Chromatography coupled to UV-Visible Detector (HPLC-UV), Liquid Chromatography coupled to High-Resolution Mass Spectrometry (LC-HRMS), Liquid chromatography-mass spectrometry (LC-MS), Liquid chromatography-mass spectrometry coupled with mass spectrometry (LC-MS/MS), High-Resolution Mass Spectrometry coupled with ultraviolet detector and Liquid Chromatography (LC-UV-HRMS), Ultra-High Performance Liquid Chromatography coupled to a Diode Array Detector (UHPLC-DAD), Ultra-High Performance Liquid Chromatography coupled to Mass Spectrometry (UHPLC-MS), Ultra-High Performance Liquid Chromatography coupled to a Diode Detector and Mass Spectrometer (UPLC-DAD-MS), Ultra-High Performance Liquid Chromatography with High-Resolution Mass Spectrometry and Tandem Mass Spectrometry (UPLC-HR-MS/MS) and Ultra Performance Liquid Chromatography coupled to UV-Visible Detector (UPLC-UV).

The publications were analyzed separately, and the studies selected for this review were categorized using codes from 1 to 44. The results are presented in tables, primarily aiming to demonstrate the aspects identified as most relevant, according to the objectives of this study.

3. Results and Discussion

3.1. Overview of Antibiotic-Producing Bacteria and Study Characteristics

After reviewing the selected bibliographic material, information was extracted

that answered the guiding research question: "What are the main strategies for obtaining, cultivating, and processing bacteria that produce antibiotic substances against *Staphylococcus aureus* and *Pseudomonas aeruginosa* described in the literature, considering their sources of isolation, taxonomic diversity, growth conditions, and methodologies for extracting and identifying bioactive molecules?" The overview of the articles found, in relation to the species that produce antibiotic substances and their sources of isolation, and target species, is presented in **Table S1**.

3.1.1. Genera of Antibiotic-Producing Bacteria and Target Pathogens

Among the target species evaluated in the included studies (**Table S2**), *Staphylococcus aureus* was the most frequently investigated, appearing in 32 out of 44 studies (72.7%). Some studies simultaneously analyzed *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with 9 out of 44 studies (20.5%), while only a small number of articles focused exclusively on *Pseudomonas aeruginosa*, representing 3 out of 44 studies (6.8%). This overview shows that, although both species are relevant pathogens, there is a predominance of studies focused on the potential use of bacterial supernatants in prospecting for molecules with activity against *S. aureus*, while *P. aeruginosa* remains relatively unexplored, representing a gap and, at the same time, a promising opportunity for future investigations.

Regarding the genera of bacteria that produce bioactive substances, there is a predominance of the genus *Bacillus*, one of the bacterial genera most associated with its ability to produce enzymes and proteins [36], with 13 species belonging to this genus being used in the studies (**Table S3**). Furthermore, there is a predominance of lactic acid bacteria genera, which includes genera such as *Brevibacillus, Enterococcus, Lacticaseibacillus, Lactiplantibacillus, Lactobacillus, Lactococcus, Ligilactobacillus, Loigolactobacillus, Pediococcus,* and *Vagococcus*.

Among the species of the genus *Bacillus*, the one that stood out the most was *Bacillus subtilis*, present in 3 studies. *B. subtilis* is a Gram-positive bacterium that forms heat-resistant spores as part of its life cycle and is reported as a fast-growing bacterium. Its genetic material can also be manipulated due to its ability to acquire external DNA and integrate it into its genome, making this species one of the most studied bacteria for this type of function, along with *Escherichia coli* [37].

Because it is a non-pathogenic, versatile, and easy-to-cultivate bacterium, *B. subtilis* can be used for various purposes, such as the production of traditional foods in Asia and the production of vitamins, amino acids, and enzymes [37]. Due to the many applications of *B. subtilis* being associated with its ability to produce and secrete proteins, research has focused on its secretome, which includes both its protein secretion machinery and the proteins themselves [36].

These findings demonstrate the importance of bioprospecting as a methodology for obtaining bioactive molecules, given the variety of species found that can be used for this purpose. Li and collaborators [38] used species from the genera *Lactiplantibacillus, Lactobacillus*, and *Ligilactobacillus*, belonging to the lactic acid bacteria group, from different foods, obtaining bioactive molecules against *P.*

aeruginosa. On the other hand, studies have used species that are not part of the lactic acid bacteria group, such as *Staphylococcus pseudointermedius*, isolated from the raccoon dog, which has shown the ability to produce molecules against *S. aureus*.

3.1.2. Sources of Isolation

Of all the reported sources of bacterial isolation, the marine environment was the most frequently cited, appearing in 13 studies (**Table S4**). This environment, especially bacteria isolated from marine sponges, has been shown in recent research to be a rich source of substances with diverse biological actions, particularly in relation to antibiotic or antibiofilm molecules [39], but also for applications in the energy, food, nutraceutical and biorefinery industries [3] [40] [41].

Food and soil are also explored sources, with 8 studies each obtaining species from these locations. Food production, for example, is closely linked to the relationship between microorganisms and plants, given that microorganisms present in the soil are able to participate in nutrient cycling and waste processing through the enzymes they produce, and such enzymes can be the target of bioprospecting processes [42]. Garcia-López and collaborators [43] used strains of *Lactiplantibacillus paraplantarum* and *Pediococcus acidilactici* isolated from sausages, finding activity against *Listeria monocytogenes, Escherichia coli, Clostridium perfringens*, and *Staphylococcus aureus*.

Among the sources investigated in the analyzed studies, the human microbiota remains comparatively underexplored as a source for antimicrobial-producing microorganisms, despite its recognized microbial diversity and biotechnological potential. Notably, only one study included in this review reported the use of intact human skin as a source for bioprospecting. The skin microbiome represents a highly diverse and dynamic ecological niche, shaped by continuous interactions between microorganisms and the host across one of the largest and most exposed surfaces of the human body. This unique ecological context suggests substantial, yet still largely untapped, potential for the discovery of new antibiotic substances. Therefore, expanding bioprospecting efforts toward the human microbiota may represent a promising direction for future research aimed at identifying new antimicrobial-producing microorganisms [44].

The resident skin microbiota is capable of producing antimicrobial peptides that, in the normal functioning of the human body, have the function and capacity to combat the establishment of external species at that site. Among these antimicrobial peptides, bacteriocins demonstrate this ability to inhibit pathogenic microorganisms in *in vitro* and *in vivo* studies [45]. Due to the need to discover new compounds with antibacterial activity to control the spread of antibiotic-resistant microorganisms, interest in the protective role played by the skin microbiota has increased. In the study by Pedretti and collaborators [44], a strain of *Bacillus siamensis* was isolated from intact skin and showed the ability to produce molecules with antimicrobial activity against several pathogenic species, such as *Staphylococcus aureus, Enterococcus faecalis, Streptococcus agalactiae,* and *Candida* spp.

3.2. Methods for Bacterial Culture, Extraction and Identification of Bioactive Molecules

Table S5 summarizes the approaches used across studies for the discovery and characterization of antibiotic molecules, including extraction strategies, cultivation conditions, and the general types of analytical methods applied for compound identification. Collectively, this synthesis highlights the methodological diversity employed to obtain and characterize antibiotic substances with activity against antibiotic-resistant bacteria.

3.2.1. Culture Media

The main culture media used were Man, Rogosa and Sharpe (MRS) broth, used in 11 studies, and Luria-Bertani (LB) broth, used in 9 studies (Table S6). MRS broth, considered a rich culture medium, is the standard choice for the isolation of lactic acid bacteria. It uses glucose as a carbon source and various nitrogen sources in its composition, including yeast extract, meat extract, and peptone, and is supplemented with minerals that provide micronutrients [46] [47]. LB broth is a complex culture medium containing tryptone, yeast extract, and NaCl [48]. It is noted as one of the main culture media for bacteria, being a good source of energy for the growth of *Lactobacillus* and other lactic acid bacteria [49].

3.2.2. Incubation Temperature

The most frequent incubation temperatures were 37˚C and 30˚C, with 15 and 12 studies, respectively (Table S7). Each microorganism is adapted to specific temperature conditions for its cultivation, as evidenced by the work of Lei and collaborators [50], in which the species *Lactobacillus plantarum* isolated from human feces was cultivated at 37˚C, the temperature corresponding to its habitat at the time of collection and isolation. In contrast, the study by Shirazi and collaborators [51], which isolated the species *Laceyella sacchari*, *Thermoactinomyces* sp., and *Laceyella* sp. from the surroundings of volcanoes and other locations, cultivated these species at 50˚C, given that they are thermophilic microorganisms.

3.2.3. Incubation Times

The most frequently used incubation times were 24 and 48 hours, with 14 and 9 studies each, respectively (Table S8). In the work of Levenfors and collaborators [52], the incubation time varied between 4 and 7 days, in an attempt to evaluate the conditions under which the production of bioactive substances was optimized. This practice, along with variations in other cultivation parameters, can help determine the best yields for the production of substances of interest.

3.2.4. Shaking during Bacterial Growth

Regarding the use of shaking during the incubation of the culture medium containing the inoculum, 26 studies used this technique, while 15 did not (Table S9). During their growth, bacterial cultures in broth that are under agitation tend to have a higher growth rate, which can be attributed to the aeration of the medium due to the agitation process [53]. Therefore, this practice should be considered for

experiments involving bioprospecting, when applicable, but taking into account the most suitable conditions for obtaining the bioactive substance.

3.2.5. Oxygen Availability

The most frequently observed oxygen availability in the studies was aerobic, with 34 studies using this incubation condition and 7 studies using anaerobic conditions (**Table S10**). The species cultivated in the absence of oxygen were *Bacillus* spp., *Bacillus subtilis*, *Lacticaseibacillus rhamnosus,* and *Lactobacillus plantarum*. These species are facultative anaerobes and can be cultivated under such conditions, especially if the biotechnological direction of their production of bioactive substances is promoted.

3.2.6. Extraction Methods

Among the methodologies used for the extraction of antibiotic molecules from bacterial culture supernatants, the organic solvent extraction method is the most widely used, being cited in 19 studies (**Table S11**).

The selection of molecules by different polarities allows the separation of the various components originating from bacterial metabolism [54], enabling phenotypic tests with smaller sets of molecules or even the isolated molecule. This approach is also important for the subsequent identification of molecules, since it makes it possible to know with greater certainty the bioactive molecule among several molecules produced by that species [55].

Another methodology used is ammonium sulfate precipitation, aiming at the separation of proteins present in the cell-free supernatant (CFS). This method was used in 13 studies and is based on the salting-out process, associated with the decrease in protein solubility when the salt concentration in the solution increases, allowing an increase in extraction yield and optimizing the protein purification process [56].

3.2.7. Identification Methods

After extracting the molecules of interest from their original CFS, another important step is the structural identification of that compound. The main methods found were Nuclear Magnetic Resonance (NMR), used in 10 studies, in addition to several methodologies based on chromatographic methods followed by mass spectrometry (**Table S12**).

In the studies included in this review, nuclear magnetic resonance (NMR) spectroscopy was primarily used for the structural elucidation of purified antibiotic substances obtained after extraction and preliminary fractionation steps. By providing one-dimensional spectra based on hydrogen spin analysis, NMR enabled detailed structural characterization, supporting compound identification with high reliability. In addition to its analytical robustness, NMR was frequently valued for being non-destructive, allowing sample recovery after analysis [57].

Mass spectrometry (MS) was widely applied for the detection and preliminary identification of bioactive molecules in complex biological extracts. In most studies, MS was coupled with separation techniques such as high-performance liquid

chromatography (HPLC) or liquid chromatography (LC) to reduce sample complexity prior to ionization, commonly through electrospray ionization (ESI). This combined approach improved the detection of antimicrobial metabolites produced by the studied microorganisms [58]-[62].

Chromatographic techniques, particularly LC and GC, were consistently used as essential preparatory and separation steps before MS or, in some cases, NMR analysis. These methods enabled the fractionation of crude extracts and facilitated the isolation of antibiotic substances. In quantitative applications, LC-MS workflows frequently relied on targeted analyses using standards, although a recurring limitation across studies was the scarcity of appropriate reference compounds for absolute quantification. To address this, some studies employed isotopically labelled standards or metabolic labelling strategies to improve quantification accuracy in complex mixtures [63] [64].

4. Conclusions

This integrative review provides a consolidated overview of methodological strategies used for the isolation, cultivation, extraction, and identification of antibiotic-producing bacteria active against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Rather than focusing on isolated experimental outcomes, the synthesis highlights patterns in antimicrobial bioprospecting, including the convergence of a limited number of environmental sources, the recurrent use of specific cultivation media, and the predominance of established analytical workflows for compound characterization.

A key contribution of this review is the identification of research biases and knowledge gaps in the current literature. Although marine environments, soil, and food are the most frequently explored sources, this prevalence reflects historical research trends rather than confirmed superiority in antimicrobial yield. Notably, the human microbiota remains significantly underrepresented, despite its ecological diversity and strong potential as a source of new microorganisms for bioprospecting. In addition, research efforts are more concentrated on *S. aureus*, while *P. aeruginosa*, a critical WHO priority pathogen, receives less attention in terms of bioprospecting strategies and microbial sources.

Overall, the reviewed studies demonstrate shared methodological frameworks in cultivation and compound recovery, particularly the use of nutrient-rich media, specific incubation periods, and conventional extraction approaches, which may limit the discovery of less readily cultivable or slow-growing producers. Similarly, reliance on established analytical pipelines such as chromatography, NMR, and mass spectrometry reinforces the identification of known compound classes, potentially limiting chemical novelty.

These findings emphasize the need to diversify both ecological sources and methodological approaches in future studies, particularly by expanding investigations into underexplored microbiomes such as the human-associated microbiota and by strengthening targeted efforts against *P. aeruginosa*. In this sense, the pre-

sent review not only compiles existing methodologies but also highlights critical gaps that can guide more innovative and discovery-oriented bioprospecting strategies. By integrating these perspectives, this synthesis contributes to the advance of antimicrobial research aimed at addressing the global challenge of multidrug resistance.

A limitation of this review is the exclusive use of PubMed/MEDLINE, which may have resulted in the exclusion of relevant studies indexed in other databases, such as Scopus, Web of Science, and Embase. Additionally, study selection and data extraction were conducted by a single reviewer, which may increase the risk of selection bias or data extraction errors. The absence of a formal methodological quality assessment may limit the comparison of the robustness of the included studies.

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Author Contributions

Gabriel Luis Cavalcanti Valente: Conceptualization; Methodology; Investigation; Formal analysis; Data curation; Visualization; Writing—original draft; Writing—review & editing.

Francislene Juliana Martins: Methodology; Investigation; Data curation; Writing—review & editing.

Geraldo Renato de Paula: Formal analysis; Validation; Visualization; Writing—review & editing.

Adriene Ribeiro Lima: Conceptualization; Supervision; Resources; Project administration; Funding acquisition; Writing—review & editing.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplement

Table S1. Identification of the articles evaluated, types of bacteria producing antibiotic substances, sources of isolation and target species of the studies.

N	First author	Year	Article's title	Biomolecule-producing species	Source of isolation	Target species of the study
1	Pedretti [44]	2024	Cell-Free Supernatant from a Strain of *Bacillus siamensis* Isolated from the Skin Showed a Broad Spectrum of Antimicrobial Activity.	*Bacillus siamensis*	Intact skin	*Staphylococcus aureus, Enterococcus faecalis, Streptococcus agalactiae, Candida spp.*
2	Ogunsile [15]	2023	Anti-Methicillin-Resistant *Staphylococcus aureus* and Antibiofilm Activity of New Peptides Produced by a *Brevibacillus* Strain.	*Brevibacillus* sp.	Soil	*Staphylococcus aureus*
3	Songnaka [65]	2022	Purification and Characterization of Novel Anti-MRSA Peptides Produced by *Brevibacillus* sp. SPR-20.	*Brevibacillus* sp.	Soil	*Staphylococcus aureus*
4	Kaweewan [66]	2020	Isolation and Structure Determination of a New Antibacterial Peptide Pentaminomycin C from *Streptomyces cacaoi* subsp. *cacaoi*.	*Streptomyces cacaoi subsp. cacaoi*	Food (cocoa)	*Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus*
5	Lin [67]	2021	Multi-Omics Analysis Reveals Anti-*Staphylococcus aureus* Activity of Actinomycin D Originating from *Streptomyces parvulus*.	*Streptomyces parvulus*	Marine environment (coral)	*Staphylococcus aureus*
6	Kokkini [68]	2022	Exploring *Micromonospora* as Phocoenamicins Producers.	*Micromonospora* sp.	Marine environment	*Staphylococcus aureus, Mycobacterium tuberculosis, Mycobacterium bovis*
7	Zhang [69]	2023	Two Antimicrobial Peptides Derived from *Bacillus* and Their Properties.	*Bacillus* spp.	Food	*Staphylococcus aureus, Bacillus cereus, Salmonella enterica*
8	Suaifan [70]	2023	Antibiotic—*Lysobacter enzymogenes* Proteases Combination as a Novel Virulence Attenuating Therapy.	*Lysobacter enzymogenes*	Data not provided	*Staphylococcus aureus, Escherichia coli*
9	Levenfors [52]	2020	Antibacterial Pyrrolidinyl and Piperidinyl Substituted 2,4-diacetylphloroglucinols from *Pseudomonas protegens* UP46.	*Pseudomonas protegens*	Soil	*Staphylococcus aureus, Bacillus cereus*
10	Santos [7]	2022	Antimicrobial Activity of Supernatants Produced by Bacteria Isolated from Brazilian Stingless Bee's Larval Food.	*Staphylococcus epidermidis, Providencia rettgeri, Enterococcus faecalis, Vagococcus fluvialis, Serratia marcescens*	Animal (stingless bees)	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*
11	Romero-	2023	Genomic and Phenotypic	*Pseudomonas* sp.	Marine	*Staphylococcus aureus*

	González [71]		Characterization of *Pseudomonas* sp. GOM7, a Novel Marine Bacterial Species with Antimicrobial Activity against Multidrug-Resistant *Staphylococcus aureus*.		environment	
12	Gharaei [72]	2022	Isolation, Optimization, and Structural Characterization of Glycolipid Biosurfactant Produced by Marine Isolate *Shewanella algae* B12 and Evaluation of Its Antimicrobial and Anti-Biofilm Activity.	*Shewanella algae*	Marine environment	*Bacillus cereus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Acinetobacter* sp.
13	Tariq [73]	2023	Agonistic Antibacterial Potential of *Loigolactobacillus coryniformis* BCH-4 Metabolites against Selected Human Pathogenic Bacteria: An *in Vitro* and *in Silico* Approach.	*Loigolactobacillus coryniformis*	Data not provided	*Escherichia coli, Bacillus cereus, Staphylococcus aureus*
14	Ye [74]	2020	8-Deoxy-Rifamycin Derivatives from *Amycolatopsis mediterranei* S699 ΔrifT Strain.	*Amycolatopsis mediterranei*	Data not provided	*Staphylococcus aureus*
15	Ramalingam [75]	2022	Structural Characterization, Antimicrobial, Antibiofilm, Antioxidant, Anticancer and Acute Toxicity Properties of N-(2-hydroxyphenyl)-2-phenazinamine From *Nocardiopsis exhalans* (KP149558).	*Nocardiopsis exhalans*	Marine environment (Coral)	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*
16	Wei [76]	2022	Isolation and Characterization of Bacteriocin-Producing *Lacticaseibacillus rhamnosus* XN2 from Yak Yoghurt and Its Bacteriocin.	*Lacticaseibacillus rhamnosus*	Food (Yak Yoghurt)	*Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Brochothrix thermosphacta, Clostridium butyricum, Staphylococcus aureus, Listeria innocua, Listeria monocytogenes, Escherichia coli*
17	Zhu [77]	2021	Purification, Characterization, and Mode of Action of Paracin 54, a Novel Bacteriocin against *Staphylococci*.	*Lactobacillus paracasei*	Feces	*Staphylococcus aureus*
18	Mlambo [78]	2022	Bioactive Metabolites of *Lactiplantibacillus plantarum* K014 against Methicillin-Resistant *Staphylococcus aureus* ATCC43300 and *in Vitro* Evaluation of Its Antibacterial, Antioxidant and Anti-inflammatory Activities.	*Lactiplantibacillus plantarum*	Food (dairy products)	*Staphylococcus aureus*
19	Trabelsi [79]	2022	Study of the Antimicrobial Potential of Actinomycetes Isolated from Organic and Inorganic Waste.	*Actinomyces* sp.	Compost pile	*Candida albicans, Staphylococcus aureus, Acinetobacter baumannii,*

						Pseudomonas aeruginosa, Escherichia coli
20	Yang [80]	2020	Antibiotic Angucycline Derivatives from the Deepsea-Derived *Streptomyces lusitanus*.	*Streptomyces lusitanus*	Marine environment	*Enterococcus faecium, Enterococcus faecalis, Staphylococcus aureus*
21	Sarjoughian [81]	2020	Bioactivity of Bac70 Produced by *Bacillus atrophaeus* Strain DDBCC70.	*Bacillus atrophaeus*	Soil (desert)	*Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus*
22	Guo [82]	2020	Mining, Heterologous Expression, Purification and Characterization of 14 Novel Bacteriocins from *Lactobacillus rhamnosus* LS-8.	*Lactobacillus rhamnosus*	Food (chinese fermented pickles)	*Escherichia coli, Staphylococcus aureus*
23	Nunes [39]	2021	Antimicrobial and Antibiofilm Activities of Marinesponge-Associated Bacteria against Multidrug-Resistant *Staphylococcus* spp. Isolated from Canine Skin.	*Bacillus algicola, Bacillus circulans, Bacillus pumilus, Bacillus* sp., *Kocuria* sp., *Pseudomonas denitrificans, Pseudomonas fluorescens, Pseudomonas putida, Pseudovibrio ascidiaceicola, Pseudovibrio denitrificans, Pseudovibrio* sp.	Marine environment (sponge)	*Staphylococcus pseudintermedius, S. schleiferi, S. aureus, S. sciuri, S. cohnii, S. simulans, S. auricularis, S. capitis, S. epidermidis, Staphylococcussp.*
24	Yoshimura [83]	2024	Five New Analogs of Streptogramin Antibiotic Viridogrisein Isolated from *Streptomyces niveoruber*.	*Streptomyces niveoruber*	Data not provided	*Staphylococcus aureus*
25	Chakraborty [84]	2022	Bacillibactin Class of Siderophore Antibiotics from a Marine Symbiotic *Bacillus* as Promising Antibacterial Agents.	*Bacillus amyloliquefaciens*	Marine environment (algae)	*Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae*
26	Cavanaugh [85]	2021	*Exiguobacterium* sp. Is Endowed with Antibiotic Properties against Gram Positive and Negative Bacteria.	*Exiguobacterium* sp.	Marine environment (pond)	*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*
27	Mousavi [86]	2023	Antibacterial Properties of Bacteriocin Purified from *Serratia marcescens* and Computerized Assessment of its Interaction with Antigen 43 in *Escherichia coli*	*Serratia marcescens*	Shrimp farming tank	*Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Vibrio fischeri, Vibrio harveyi*
28	Silva [87]	2022	Insights into the Antimicrobial Activities and Metabolomes of Aquimarina (Flavobacteriaceae, Bacteroidetes) Species from the Rare Marine Biosphere.	*Sarcotragus spinosulus, Ircinia variabilis, Eunicella labiata, Aquimarina muelleri, Aquimarina*	Marine environment	*Staphylococcus aureus*

				spongiae, *Aquimarina latercula*		
29	Naveed [88]	2023	Purification, Characterization and Bactericidal Action of Lysozyme, Isolated from *Bacillus subtillis* BSN314: A Disintegrating Effect of Lysozyme on Gram-Positive and Gram-Negative Bacteria.	*Bacillus subtilis*	Data not provided	*Bacillus subtilis*, *Micrococcus luteus*, *Bacillus cereus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*
30	Wang [89]	2020	Hetiamacin E and F, New Amicoumacin Antibiotics from *Bacillus subtilis* PJS Using MS/MS-Based Molecular Networking.	*Bacillus subtilis*	Soil (desert)	*Staphylococcus epidermidis*, *Staphylococcus aureus*
31	Mao [90]	2023	Impact of Cell-Free Supernatant of Lactic Acid Bacteria on *Staphylococcus aureus* Biofilm and Its Metabolites.	Bactérias ácido-láticas	Cow milk	*Staphylococcus aureus*
32	Hioki [91]	2021	Heterologous Production of Active form of Beta-Lytic Protease by *Bacillus* subtilis and Improvement of Staphylolytic Activity by Protein Engineering.	*Bacillus subtilis*	Data not provided	*Staphylococcus aureus*
33	Zammuto [92]	2023	Lichenysin-Like Polypeptide Production by *Bacillus licheniformis* B3-15 and Its Antiadhesive and Antibiofilm Properties.	*Bacillus licheniformis*	Marine environment (hot springs)	*Staphylococcus aureus*, *Pseudomonas aeruginosa*
34	Gu [93]	2022	Isolation, Identification and Characterization of Two Kinds of Deep-Sea Bacterial Lipopeptides Against Foodborne Pathogens.	*Bacillus* sp.	Marine environment	*Staphylococcus aureus*, *Pseudomonas aeruginosa*
35	Dai [94]	2021	A Novel Bacteriocin from *Lactobacillus pentosus* ZFM94 and Its Antibacterial Mode of Action.	*Lactobacillus pentosus*	Feces	*Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*
36	Yi [31]	2024	Human Milk-Derived Enterococcus faecalis HM20: A Potential Alternative Agent of Antimicrobial Effect against Methicillin-Resistant *Staphylococcus aureus* (MRSA).	*Enterococcus faecalis*	Human milk	*Staphylococcus aureus*
37	Madoromae [95]	2025	Investigating the Production and Synergistic Antibacterial Activity of Bacteriocin-Like Substance from *Brevibacillus laterosporus* SA-14 (TISTR 2453) for Enhanced Wound Healing.	*Brevibacillus laterosporus*	Air	*Staphylococcus aureus*
38	Baranova [96]	2024	Bacteriocin from the Raccoon Dog Oral Microbiota Inhibits the Growth of Pathogenic Methicillin-Resistant *Staphylococcus aureus*.	*Staphylococcus pseudintermedius*	Animal (racoon dog)	*Staphylococcus aureus*
39	Li [38]	2023	Screening and Metabolomic Analysis	*Lactiplantibacillus*	Food	*Pseudomonas aeruginosa*

			of Lactic Acid Bacteria-Antagonizing *Pseudomonas aeruginosa*.	*plantarum, Lactobacillus acidophilus, Lactobacillus gasseri, Ligilactobacillus salivarius*	(Fermented milk, pickles, Xizang's kefir, fermented rice)	
40	Kaya [97]	2020	Characterization of Pediococcus acidilactici PFC69 and Lactococcus lactis PFC77 Bacteriocins and Their Antimicrobial Activities in Tarhana Fermentation.	*Pediococcus acidilactici, Lactococcus lactis*	Food (Tarhana)	*Bacillus cereus, Staphylococcus aureus*
41	García-López [43]	2023	*Lactiplantibacillus paraplantarum* BPF2 and *Pediococcus acidilactici* ST6, Two Bacteriocinogenic Isolated Strains from Andalusian Spontaneous Fermented Sausages.	*Lactiplantibacillus paraplantarum, Pediococcus acidilactici*	Food (fermented sausages)	*Listeria monocytogenes, Escherichia coli, Clostridium perfringens, Staphylococcus aureus*
42	Bano [98]	2022	Bioprospecting of the Novel Isolate *Microbacterium proteolyticum* LA2(R) from the Rhizosphere of Rauwolfia Serpentina.	*Microbacterium proteolyticum*	Plants and soil	*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella abony*
43	Lei [50]	2020	Partial Purification and Characterization of Abroad-Spectrum Bacteriocin Produced by a *Lactobacillus plantarum* Zrx03 Isolated from Infant's Feces.	*Lactobacillus plantarum*	Feces	*Staphylococcus aureus, Bacillus subtilis, Bacillus anthracis, Escherichia coli, Salmonella* sp.
44	Shirazi [51]	2023	Isolation and Screening of *Thermoactinomycetaceae* Family Members as an Extremophilic Poor Investigated and Promising Natural Source of Antimicrobial Substances.	*Laceyella sacchari, Thermoactinomyces* sp., *Laceyella* sp.	Soil	*Staphylococcus aureus*

Table S2. Target species of the studies.

Target species of the studies	Number of appearances	Percentage of total
Staphylococcus aureus	32	72.7%
Pseudomonas aeruginosa	3	6.8%
Both species	9	20.5%

Table S3. Biomolecule-producing bacterial genera.

Biomolecule-producing bacterial genera	Number of appearances	Percentage of total
Aquimarina	3	4.3%
Bacillus	13	18.6%
Lactobacillus	7	10.0%
Streptomyces	4	5.7%
Actinomyces	1	1.4%

Continued

Amycolatopsis	1	1.4%
Brevibacillus	3	4.3%
Enterococcus	2	2.9%
Eunicella	1	1.4%
Exiguobacterium	1	1.4%
Ircinia	1	1.4%
Kocuria	1	1.4%
Laceyella	2	2.9%
Lacticaseibacillus	1	1.4%
Lactiplantibacillus	3	4.3%
Lactococcus	1	1.4%
Ligilactobacillus	1	1.4%
Loigolactobacillus	1	1.4%
Lysobacter	1	1.4%
Microbacterium	1	1.4%
Micromonospora	1	1.4%
Nocardiopsis	1	1.4%
Pediococcus	2	2.9%
Providencia	1	1.4%
Pseudomonas	5	7.1%
Pseudovibrio	3	4.3%
Sarcotragus	1	1.4%
Serratia	2	2.9%
Shewanella	1	1.4%
Staphylococcus	2	2.9%
Thermoactinomyces	1	1.4%
Vagococcus	1	1.4%

Table S4. Source of isolation of the biomolecule-producing species.

Source of isolation of the biomolecule-producing species	Number of appearances	Percentage of total
Food	8	16.7%
Marine environment	13	27.1%
Animal or insect	2	4.2%
Data not provided	6	12.5%
Feces	4	8.3%

Continued

Soil	8	16.7%
Air	1	2.1%
Cow milk	1	2.1%
Human milk	1	2.1%
Intact human skin	1	2.1%
Compost pile	1	2.1%
Plants	1	2.1%
Shrimp farming tank	1	2.1%

Table S5. Identification methods, nature of the identified bioactive molecule, target species, extraction method used, and cultivation conditions adopted for the production of antibiotic molecules.

N°	Identification method	Nature of the identified bioactive molecule	Target species (between *S. aureus* and *P. aeruginosa*)	Extraction method	Culture medium	Incubation temperature	Incubation time	Shaking during incubation	Oxygen availability during growth
1	Data not provided	Data not provided	*S. aureus*	Data not provided	TSB broth	37˚C	24 h	No	Aerobiosis
2	CIEX, RPC, LC-MS/MS, SDS-PAGE	Peptide	*S. aureus*	Precipitation by ammonium sulfate	LB broth	30˚C	24 h	Yes	Aerobiosis
3	CIEX, RPC, LC-MS/MS	Peptide	*S. aureus*	Precipitation by ammonium sulfate	LB broth	30˚C	24 h	Yes	Aerobiosis
4	HPLC, ESI-MS, RMN	Peptide	*S. aureus*	Solvent extraction (Methanol)	Data not provided	30˚C	9 days	No	Aerobiosis
5	RMN, MS	Actinomicin D	*S. aureus*	Silica gel chromatography followed by HPLC	Data not provided	Data not provided	Data not provided	Data not provided	Data not provided
6	LC-HRMS, LC-UV-HRMS	Focoenamycin, Maklamycin	*S. aureus*	Solvent extraction (acetone)	ATCC-2 broth	28˚C	7 days	Yes	Aerobiosis
7	IEX, RP-HPLC, LC-MS/MS	Peptide	*S. aureus*	Precipitation by ammonium sulfate	NB broth	30˚C	24 h	Yes	Anaerobiosis
8	Data not provided	Data not provided	*S. aureus*	Data not provided	TSB broth	28˚C	11 days	Yes	Aerobiosis
9	HPLC-MS, UHPLC-MS	Pyrrolnitrine, pyoluteorin and2,4-diacetylphloroglucinol	*S. aureus*	Solvent extraction (acetonitrile)	Modified mineral medium and tryptone broth	20˚C	4 - 7 days	Yes	Aerobiosis
10	Data not provided	Data not provided	*P. aeruginosa, S. aureus*	Data not provided	LB broth	31˚C	48 h	Yes	Aerobiosis

11	Data not provided	Data not provided	*S. aureus*	Solvent extraction (n-hexane, chloroform, ethyl acetate)	LB broth	30˚C	48 h	No	Aerobiosis
12	GC-MS, FTIR, RMN	Biosurfactant	*P. aeruginosa*	Solvent extraction (ethyl acetate:Methanol 3:1)	NB broth	37˚C	96 h	Yes	Aerobiosis
13	ESI-MS/MS	Macrolide	*S. aureus*	Solvent extraction (ethyl acetate, chloroform and methanol), silica gel chromatography	MRS broth	37˚C	72 h	Yes	Aerobiosis
14	RMN	Rifampicin derivatives	*S. aureus*	Solvent extraction (ethyl acetate and Methanol)	ISP 2 broth	28˚C	Data not provided	Yes	Aerobiosis
15	GC-MS, FTIR, RMN	N-(2-hidroxi-fenil)-2-fenazinamin	*P. aeruginosa, S. aureus*	Solvent extraction (ethyl acetate), silica gel chromatographic column	ISP 2 broth	25˚C	7 days	Yes	Aerobiosis
16	SEC, UHPLC-DAD, C18 column	Data not provided	*S. aureus*	Precipitation by ammonium sulfate	MRS broth	37˚C	24 h	No	Anaerobiosis
17	RP-HPLC, CIEX	Protein	*S. aureus*	Precipitation by ammonium sulfate	MRS broth	37˚C	36 h	No	Aerobiosis
18	Data not provided	Data not provided	*S. aureus*	Data not provided	MRS broth	37˚C	24 h	No	Aerobiosis
19	HPLC-MS	Purpuromicin	*P. aeruginosa, S. aureus*	Solvent extraction (acetone and DMSO)	ATCC-2 broth	28˚C	7 days	Yes	Aerobiosis
20	HPLC-MS, RMN	Grincamycin, angucycline derivatives	*S. aureus*	Solvent extraction (ethyl acetate, Methanol and hexane) and C18 chromatographic column	Modified broth	30˚C	6 days	Yes	Anaerobiosis
21	Data not provided	Data not provided	*P. aeruginosa, S. aureus*	Precipitation by ammonium sulfate	BHI broth	30˚C	24 h	Yes	Aerobiosis
22	LC-MS/MS	Proteins	*S. aureus*	Precipitation by ammonium sulfate	MRS broth	37˚C	24 h	Yes	Anaerobiosis
23	Data not provided	Data not provided	*S. aureus*	Solvent extraction (ethyl acetate)	BHI broth and Marine broth	25˚C	48 h	No	Aerobiosis
24	LC-MS/MS e RMN	Viridogrisein, griseoviridine	*S. aureus*	Solvent extraction (Methanol) and C18 column	TSB broth	30˚C	48 h	Yes	Aerobiosis
25	HPLC, GC-	Bacillibatin C	*P. aeruginosa, S.*	Solvent extraction	Data not	30˚C	36 h	No	Aerobiosis

	MS e RMN	derivatives	*aureus*	(ethyl acetate) and gel chromatographic column	provided				
26	FIA, LC-MS	Data not provided	*P. aeruginosa, S. aureus*	Solvent extraction (ethyl acetate)	LB broth	30˚C	24 h	Yes	Aerobiosis
27	Cromatografia por coluna, RMN	Protein	*P. aeruginosa*	Precipitation by ammonium sulfate	Data not provided	Data not provided	24 h	Data not provided	Data not provided
28	UPLC-HR-MS/ MS	Peptide	*S. aureus*	Solid phase extraction	MB broth	24˚C	48 h	Yes	Aerobiosis
29	Cromatografia por coluna em gel, PAGE	Lysozyme	*P. aeruginosa*	Precipitation by ammonium sulfate	LB broth	37˚C	24 h	Yes	Aerobiosis
30	UPLC-UV, UPLC-DAD-M S e RMN	Amicoumacins	*S. aureus*	Diaion HP-20 column and elution with acetone.	Modified Gause broth	28˚C	3 days	Yes	Anaerobiosis
31	UHPLC-MS	Data not provided	*S. aureus*	Data not provided	MRS broth	37˚C	24 h	Yes	Anaerobiosis
32	SDS-PAGE	Data not provided	*S. aureus*	Data not provided	LB broth supplemented with tetracycline	30˚C	15 h	No	Aerobiosis
33	FTIR	Surfactin	*P. aeruginosa, S. aureus*	Precipitation by HCl, Solvent extraction (chloroform and methanol)	MGV broth	45˚C	48 h	Yes	Aerobiosis
34	RP-HPLC, C18 chromatographic column	Fengicin and surfactin	*P. aeruginosa, S. aureus*	Precipitation by HCl and Solvent extraction (methanol)	LB broth	28˚C	48 h	Yes	Anaerobiosis
35	SDS-PAGE, RP-HPLC	Pentocin	*S. aureus*	Precipitation by ammonium sulfate	MRS broth	37˚C	20 h	No	Aerobiosis
36	Data not provided	Data not provided	*S. aureus*	Solvent extraction (ethyl acetate)	MRS broth	37˚C	48 h	No	Aerobiosis
37	SDS-PAGE	Data not provided	*S. aureus*	Precipitation by ammonium sulfate	LB broth	37˚C	48 h	No	Aerobiosis
38	IEX	Bacteriolisins, tailocins and proteins	*S. aureus*	Solid phase extraction	Data not provided	Data not provided	Data not provided	Data not provided	Data not provided
39	Data not provided	Data not provided	*P. aeruginosa*	Data not provided	MRS broth	37˚C	24 h	Yes	Aerobiosis

40	UHPLC-DAD	Bacteriocin	*S. aureus*	Precipitation by ammonium sulfate, solid phase extraction	MRS broth and M17G broth	30˚C	18 h	No	Aerobiosis
41	IEX, RP-HPLC, LC-MS/MS	Bacteriocin	*S. aureus*	Silica gel chromatography	BHI broth	37˚C	24 h	No	Aerobiosis
42	GC-MS	Cis-vaccenic acid, octadecanoic acid, cholesta-3,5-diene and n-hexadecanoic acid	*S. aureus*	Solvent extraction (ethyl acetate and Methanol)	ISP 2 broth	28˚C	20 days	Yes	Aerobiosis
43	Data not provided	Bacteriocin	*S. aureus*	Solvent extraction (ethyl acetate, butanol, hexane, dichloromethane, trichloromethane) and Precipitation by ammonium sulfate	MRS broth	37˚C	18 h	No	Anaerobiosis
44	CCD, HPLC-UV, column chromatography	Data not provided	*S. aureus*	Data not provided	ISP 2 broth	50˚C	7 days	Yes	Aerobiosis

Table S6. Culture medium applied.

Culture medium applied	Number of appearances	Percentage of total
ATCC-2 Broth	2	4.3%
MRS broth	11	23.4%
LB broth	9	19.1%
Modified mineral medium	1	2.1%
M17G Broth	1	2.1%
Marine broth	1	2.1%
MB broth	1	2.1%
MGV broth	1	2.1%
Modified broth	1	2.1%
Modified Gause Broth	1	2.1%
NB broth	2	4.3%
Tryptone broth	1	2.1%
TSB broth	3	6.4%
ISP2 broth	4	8.5%
BHI broth	3	6.4%
Data not provided	5	10.6%

Table S7. Incubation temperature.

Incubation temperature	Number of appearances	Percentage of total
20˚C	1	2.3%
24˚C	1	2.3%
25˚C	2	4.5%
28˚C	7	15.9%
30˚C	12	27.3%
31˚C	1	2.3%
37˚C	15	34.1%
45˚C	1	2.3%
50˚C	1	2.3%
Data not provided	3	6.8%

Table S8. Incubation time.

Incubation time	Number of appearances	Percentage of total
15 hours	1	2.3%
18 hours	2	4.5%
20 hours	1	2.3%
24 hours	14	31.8%
36 hours	2	4.5%
48 hours	9	20.5%
72 hours	2	4.5%
96 hours	1	2.3%
144 hours	1	2.3%
168 hours	5	11.4%
216 hours	1	2.3%
264 hours	1	2.3%
480 hours	1	2.3%
Data not provided	3	6.8%

Table S9. Usage of shaking during incubation.

Usage of shaking during incubation	Number of appearances	Percentage of total
Yes	26	59.1%
No	15	34.1%
Data not provided	3	6.8%

Table S10. Oxygen availability during growth.

Oxygen availability during growth	Number of appearances	Percentage of total
Aerobiosis	34	77.3%
Anaerobiosis	7	15.9%
Data not provided	3	6.8%

Table S11. Biomolecule extraction methods.

Biomolecule extraction methods	Number of appearances	Percentage of total
Chromatography	9	16.7%
Solid-phase extraction	3	5.6%
Solvent extraction	19	35.2%
Hydrochloric acid precipitation	2	3.7%
Ammonium sulfate precipitation	13	24.1%
Data not provided	8	14.8%

Table S12. Biomolecule identification methods.

Biomolecule identification methods	Number of appearances	Percentage of total
Separation techniques only	20	28.2%
Hybrid techniques	24	33.8%
Spectrometric techniques	3	4.2%
Spectroscopic techniques	10	14.1%
Electrophoretic techniques	5	7.0%
Data not provided	9	12.7%