REVIEW ARTICLE

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Analysis of "1st Brazilian Recommendation for Biofilm Management in Chronic and Complex Wounds"

Análise da "1ª Recomendação Brasileira para o Gerenciamento do Biofilme em Feridas Crônicas e Complexas"

Análisis de la "1ª Recomendación Brasileña para el Manejo de Biopelículas en Heridas Crónicas y Complejas"

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ABSTRACT

Objectives: Analyze critically the "1st Brazilian Recommendation for Biofilm Management in Chronic and Complex Wounds" (from Portuguese, "1ª Recomendação Brasileira para o Gerenciamento de Biofilme em Feridas Crônicas e Complexas"). **Method:** Reviewing information contained in said document according to current literature. **Results:** The publication was showed to lack methodology compatible with its title; gaps in the recommendations were perceived regarding evidence classification, as well as an absence of grounding from important international consensus, published in the last three years, about treatment of complex wounds with suspected biofilm. **Conclusion:** The document was concluded to be inadequate for use as a clinical guideline, being considered only a bibliographic review about the theme.

DESCRIPTORS: Commentary; Guidelines for clinical practice; Nursing; Biofilm; Wound infection; Stomatherapy.

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RESUMO

Objetivos: Analisar criticamente a "1ª Recomendação brasileira para o gerenciamento de biofilme em feridas crônicas e complexas". **Método:** Realizou-se revisão da literatura atual às informações nele contidas. **Resultados:** Observou-se que a publicação carece de metodologia compatível com o título, existem lacunas nas recomendações quanto à classificação das evidências e com ausência de fundamentação a partir de importantes consensos internacionais para o tratamento das feridas complexas com suspeita de biofilme, publicados nos últimos três anos. **Conclusão:** Conclui-se que o manuscrito não deve ser usado como guia de recomendações clínicas, mas como revisão bibliográfica sobre o tema.

DESCRITORES: Comentário. Guia de prática clínica. Enfermagem. Biofilme. Infecção dos ferimentos. Estomaterapia.

RESUMEN

Objetivo: Analizar críticamente la "1ª Recomendación brasileña para el manejo de biopelículas en heridas crónicas y complejas". **Método:** Para el análisis del documento, se realizó revisión de la literatura actual a la luz de las informaciones en él referidas. **Resultados:** Fue observado que la publicación carece de metodología compatible con el título, existen lacunas en las recomendaciones en cuanto a la clasificación de las evidencias, con ausencia de fundamentación a partir de importantes consensos internacionales para el tratamiento de las heridas complejas con sospecha de biopelícula, publicados en los últimos tres años. **Conclusión:** El manuscrito no debe ser usado como guía de recomendaciones clínicas, pero como revisión bibliográfica sobre el tema.

DESCRIPTORES: Comentario. Guía de práctica clínica. Enfermería. Biopelículas. Infección de heridas. Enfermería en terapia enterostomal.

INTRODUCTION

In November 2018, a document entitled "1ª Recomendação brasileira para o gerenciamento de biofilme em feridas crônicas e complexas" ("1st Brazilian Recommendation for Biofilm Management in Chronic and Complex Wounds")[1] were published during the VII Congresso Brasileiro de Prevenção e Tratamento de Feridas (VII Brazilian Congress for Wounds Prevention and Treatment).

It represented an important initiative, given the fact that, up to then, there was not any national consensus published about said theme in Brazil. Worthy of highlighting is that a guideline of recommendation helps health professionals in the decision making to assertively manage evidence-based treatment of complex wounds.

However, after the document reading, as well as a discussion carried out by Grupo de Pesquisa em Estomaterapia: Estomias, Feridas Agudas e Crônicas e Incontinências Urinária e Anal (GPET; Research Group for Stomal Therapy: Ostomies, Acute and Chronic Wounds, and Urinary and Anal Incontinences, in English), from Escola de Enfermagem da Universidade de São Paulo, the title of the publication showed to be inconsistent with its content, since no intervention list or algorithm for management of infected wounds or with suspected biofilm were identified, although the title gave the idea of a series of recommendations. What is more, the document in discussion lacked methodology, which made it not suitable for a systematic or integrative review of the current literature, with outdated citations of classic works and omission of important international consensus published in the last three years[2–6]. Additionally, the authors of the document did not employ any method for evaluating evidences nor of validation by specialists. Finally, no specifications of Brazilian context regarding epidemiology and availability of several antimicrobial products were identified.

Considering the foretold aspects, GPET, registered in CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico; National Council for Scientific and Technological Development, in English) since 2004 and composed by trained nurses with expertise in a number of fields, including Stomal Therapy and Basic Sciences, developed this paper, employing a critical analysis approach, and aiming at helping health professionals and researchers engaged in the care for people with complex wounds, as well as at bringing to light an investigation about this theme in Brazil.

METHODS

Bearing in mind the publication of the document in discussion, a literature review took place, in search for the scientific basis of its critical analysis. The data bases employed for this purpose were PubMed and Google Scholar, with no restriction regarding the year of publication of the works

researched and limiting the languages to only English and Portuguese. The keywords employed in English were as follows: biofilms, chronic wounds, wound care, consensus, wound infection, biofilm microbiology, and prevention, alongside with Boolean operators "and" and "or" and their equivalents in Portuguese. The critical analysis focused in comparing the information contained in the object of study with the found literature, followed by the original division in themes: A. Biofilm microbiology; B. Main characteristics of chronic and complex wounds; C. Preventive management focusing on controlling infection; and D. Antimicrobial agents.

RESULTS AND DISCUSSION

The approach of choice was a discursive presentation of each topic, with the respective bibliographic material found, in order to justify the merging of the sections Results and Discussion.

A relevant aspect common to all the topics to be presented is the shortage of high quality works to enable an evidence-based practice[3–6]. There are scarce evidences related to biofilm recognition, as well as its diagnosis and treatment[6]. Although several algorithms for treatment have been elaborated, clinical data about those tools are still necessary, in order to evaluate the results after their implementation in the Brazilian context. What is more, there is a discrepancy related to the professionals' knowledge about biofilm and their importance in the management of chronic non-healing wounds[4].

A. Biofilm microbiology

With respect to biofilm microbiology, the presented review does not appear to be sufficiently comprehensive and up-to-date. Historical facts were mentioned without elucidating the context. An example is the presentation of the studies in odontology in order to substantiate biofilm management in chronic wounds, making appropriate, for better clarification, to address the history and evolution of the knowledge about bacteria, their shapes, and the advent of the first evidences for their identification in a clinical environment.

The bacterial phenotype in biofilm is scientifically discussed by organizations such as American Society for Microbiology since 1993[7], which states the extreme importance of this theme. It is fundamental to consider biofilm as a social arrangement of microbial cells enveloped by a matrix of extracellular polymeric substances (EPS) organized through the quorum sensing, which is, in its turn, formed by bacterial adhesion process[8,9]. It is known that microorganisms rarely live in colonies of only one species, meaning they live in communities[10]. Bacteria can promptly colonize solid surfaces in contact with water, either in natural or artificial environments, being able, also, of growing in planktonic form (free) or sessile clusters (adhered), considered important for biofilm formation[10–12].

Therefore, biofilm begins to form when a planktonic bacterium lodges to a surface[13], a process little explained in the document of discussion. This fixation process is caused by Brownian, or flagellar, motion, surpassing the repulsive electrostatic forces between substrate and bacterial surface. The anchoring among the bacteria takes place through cellular adhesion structures known as pili. The type of biofilm to be formed will depend on the environment to which the bacteria will adhere to form their microcolonies.

In this phase, the bacteria are enveloped by a protective matrix, and begin to express their biofilm phenotype[14–16]. It is then that bacterial hydrophobicity reduces the repulsion between extracellular matrix and bacteria[17]. Oppositely to initial biofilm, the mature biofilm is formed by microcolonies in EPS, composed of extracellular deoxyribonucleic acid (DNA), polysaccharides, proteins, and amyloid fiber, which allows the maintenance of nutrients and orchestrate the gradients of oxygen and nitric oxide from the matrix[9,11,18].

Another important feature concerns the little discussed bacterial heterogeneity[19,20]. Biofilm composition may vary according to the time of wound formation, to the kind of lesion, to the bacterial types present in the biofilm e to the target audience analyzed, aspects absent or little addressed in the document of discussion. What is more, the strategies for detecting the bacteria in the biofilm must be conducted in a manner that produces reliable outcomes. Most chronic wounds (78%) sport biofilm with important heterogeneity[21]. In patients with lesions in the feet caused by diabetes mellitus, studies show presence of mixed bacterial colonies, with both aerobic and anaerobic organisms[22–25], indicating social activity among the bacteria (sociomicrobiology)[8].

This polymicrobial arrangement assures biofilm maintenance. The most common aerobic bacterium engaged in biofilm formation in chronic wounds is *Staphylococcus aureus*, followed by *Pseudomons* spp. and *Eschericha coli*[22–25], as well as the anaerobic methicillin resistant *S. aureus* (MRSA)[26]. Among anaerobic bacteria, *Bacteroides fragilis* and *Clostridium* spp.

can be named[26–28]. It appears likely that those bacteria proliferation occurs due to tissue oxygen consumption by aerobic bacteria[29]. Therefore, for a patient with diabetes mellitus, it is necessary to perform, as standard procedures, the screening of the wound with techniques appropriate to diagnose biofilm, as well as antibiogram, in order to devise effective treating strategies[30].

B. Main characteristics of chronic and complex wounds

According to the analyzed document[1], it is known that chronic wounds are a worldwide public health issue, substantially affecting morbimortality and treatment costs. However, the document does not provide data about global prevalence and incidence of this kind of wound, and its estimates are inconsistent, once they vary according to the concept employed and the conditions considered regarding chronicity[31,32].

The definition of complex chronic wound varies. In Brazil, the concept being applied more frequently refers to wounds lasting more than three months in which there are infection, nonviable tissues, disordered healing process and association with systemic pathologies, such as diabetes mellitus and vasculitis[33]. However, in a consensus published in 2018, specialists defined as chronic wounds lesions that do not evolve with a normal healing process and may have the process jeopardized by the presence of underlying diseases[34].

In what regards to features, chronic wounds are characterized by a prolonged inflammatory process, displaying elevated cytokines (IL-1β, TNF-α), high concentration of metalloproteinases (MMP2, MMP8, MMP9) and an excess of neutrophils[35]. Other factors involved, such as the production of bacterial toxins that contribute to de depletion of collagen, the patient's nutritional status, age and immunosuppression, drugs currently being administrated, and simultaneous diseases, may influence the wound's chronicity[36].

Concerning the prevalence of chronic wounds, both in Brazil and the world, it is worthy highlighting the scarcity of comparable epidemiologic data, as well as the existence of an immense variation in published studies regarding the theme[37]. In a recent systematic review[32], the authors revealed the prevalence of lower limb ulcers, lesions caused by pressure and ulcers in patients suffering from diabetes mellitus, which displays an estimated prevalence of 1.51 ulcer per leg per thousand habitants and 2.21 ulcers of several etiologies per thousand habitants. In what regards to age, most studies describe higher indexes in patients ranging from 70 to 80 years. In Brazil, two studies, also recent, show prevalences from 5 and 10.3% for lesions caused by pressure, and from 8.5 and 3.2% for diabetic ulcers in inpatients[38] and patients undergoing primary attention[39], respectively.

Still addressing the limited number of studies dealing with the prevalence of chronic wounds both in Brazil and the world, they appear to be carried out in specific populations, not always including wounds caused by etiologies common in developing countries, such those secondary to infectious diseases as Hansen's disease.

That said, knowing the national epidemiologic data about the lesions and the factors contributing to the healing delay, either related to microorganisms or to patient's comorbidities, is highly important both to perform a correct diagnosis approach and to apply a treatment that allows early eradication of the biofilm, aiming at an appropriate and efficient care.

C. Preventive management focusing on controlling infection

In this sub-item, the analyzed document shows some important limitations that compromise its use as a single bibliographic material of reference and impose discrepancies between literature and the document's contents.

Snyder[40] considers that associating the presence of biofilm to the delay of the healing process, even when its management is appropriate, is controversial and depends on the outcomes of the ongoing researches. The document in discussion, oppositely to the aforementioned author's opinion[40], states that biofilm is visible to the naked eye when this is not 100% scientifically substantiated. The diagnosis is, primarily, clinical and must account for the presence of several clinical signals[4,5,41].

Another misconception refers to a study carried out by Rhoads[42], in which the water treatment in health institutions and the cleaning of the dressing materials are discussed as strategies for preventing infection. However, the document's author does not address this theme, focusing instead on the use of dressings with

antimicrobials, antiseptic and the like (lactoferrin, iodine, honey, xylitol, gallium etc.).

Additionally to the mentioned aspects, considered crucial in compromising the reliability of the document in question, the authors discussed several topics that do not fit in the proposed content of the sub-item, such as diagnosing techniques and biofilm characterization, suitable for treating wounds already infected, not for preventing them, although the intention was to address aspects of prevention, focusing on infection control. Some practices, such as debridement, were referred separately, not denoting the systematic approach needed for appropriately treating the wound as displayed in the 2016 World Union of Wound Healing Societies (WUWHS)[5] positioning document and in the International Wound Infection Institute (IWII)[2] consensus.

Regarding strategies to prevent biofilm formation, available literature outlines the necessity to suppress the attachment of microorganisms to the wound and, consequently, the formation of mature biofilm, which can be accomplished by appropriately cleaning the wound bed, removing nonviable tissue through debridement, and approaching the wound systematically through the strategy known for the acronym TIME (tissue, infection/inflammation, moisture balance and edge of wound)[5,6,40]. Patient-centered care must be employed, in order to improve resistance against infection, as well as moisture balance, systemic blood pressure control, and local edema, contributing to wound healing, as well as to the reduction of nutrients available for biofilm formation[2]. Table 1 displays a synthesis of the clinical recommendations for identifying and treating biofilm published to date, including further information about prevention.

Table 1. Synthesis of clinical recommendations for treating wounds with suspected biofilm.

Clinical recommendations	Source
1. Approach chronic wounds with a multimodal systematic for early diagnosis and treatment, including the simultaneous use of several therapies against biofilm, optimizing the aspects contributing for the delay of the healing process (edema, underlying diseases, nutrition, soft tissue and bone infection, pressure)[43]. Only interrupt gradually the interventions when the wound shows a stable healing pace[4,40].	Schultz, 2017[4] Snyder, 2017[40] HSE-Nolan, 2018[43]
2. Use an algorithm for diagnosing the presence of biofilm[5], which includes the recognition of following clinical indicators: therapeutic failure (topic and systemic); delay of the healing process; presence of low quality granulated tissue (brittle, hipergranulation); signs of infection > 30 days; inflammation; gelatinous material that quickly forms on the surface of the wound, despite cleaning/debridement; and great volume of exudate. In the absence of classic infection signs, consider low degree erythema as an indicator[4,41].	WUWHS-Bjarnsholt, 2016[5] Schultz, 2017[4] Keast, 2014[41]
3. Do not use microbiological culture test to diagnose the presence of biofilm, once it only indicates the presence of planktonic bacteria on the exudate/wound surface[5,40]. To perform tissue biopsy is considered gold standard for identifying biofilm[4], for more specific techniques are necessary for its identification (molecular methods based on the recognition of genetic material and techniques of rupturing polymeric matrix). In the absence of this technology, use algorithm for clinical signs of suspicion as diagnostic[4].	WUWHS-Bjarnsholt, 2016[5] Snyder, 2017[40] Schultz, 2017[4]
4. Follow a model for the wound bed preparation, prioritizing constant cleaning, removal of devitalized tissue and exudate control, disrupting the adhesion and proliferation of the biofilm on the surface of the wound. Prefer dressings that favor constant autolytic debridement, managing the exudate.	WUWHS-Bjarnsholt, 2016[5] Schultz, 2017[4] Bianchi, 2016[6]
5. Remove biofilm applying a serial debridement technique. This is one of the most important strategies for managing biofilm, yet should not be performed isolate, once it is not able to remove 100% of the biofilm, as well as being unable to prevent a new biofilm formation[5,6,40]. Follow an algorithm[44] that considers using the techniques in the following rank: mechanical, instrumental, biological-larval, autolytic or enzymatic, hydrosurgical or ultrasound, and surgical debridement. Surgical and instrumental types of debridement substantiate strong evidences of biofilm removal; autolytic, mechanical, and enzymatic types of debridement depend on the technique or product; and biological debridement shows good evidences in vitro[2]. The choice of the technique must be guided by the evaluation of the patient and through taking in consideration pros and cons. There are controversies regarding scientific evidences available about the comparison of the techniques[43]. Simultaneous use of more than one technique can improve the outcomes.	WUWHS-Bjarnsholt, 2016[5] Bianchi, 2016[6] Snyder, 2017[40] EWMA-Strohal, 2013[44] Schultz, 2017[4] IWII-Swanson, 2016[2] HSE-Nolan, 2018[43]

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Table 1. Continuation...

Clinical recommendations	Source
6. Use antiseptic solutions and antimicrobial dressings in order to reduce the microbial load (specially planktonic) and to prevent new biofilm formation after debridement[2,4–6]. Antiseptic solutions must be used to prepare the wound bed before debridement and, thus, minimize the risks of microbial translocation to deep tissues.	WUWHS-Bjarnsholt, 2016[4] Bianchi, 2016[6] Schultz, 2017[4] IWII-Swanson, 2016[2]
7. To use tools for evaluating the risk of infection in wounds can be greatly relevant for the decision making about the application of antiseptic and antimicrobial solutions[3,5]. Example: W.A.R. (Wounds at Risk), which considers the presence of comorbidities, use of immunosuppressive therapy, etiology, localization, extension and duration of the wound, status of contamination, and age and hygiene of the patient as risk factors for microbial colonizing and justification for using antimicrobial and antiseptic drugs[45].	WUWHS-Bjarnsholt, 2016[5] Kramer, 2017[3] Dissemond, 2011[45]
8. Use systemic microbial drugs in the presence of clinical signs for deep or disseminated infection, in order to reduce the concentration of planktonic bacteria in deep tissues of the wound and to prevent systemic infections[5]. There are not evidences of the systemic treatment being able to prevent or treat the biofilm in wounds[4,40]. Initiate empirical treatment with the most specific antibiotic possible for the case following the identification of the signs of infection[46]. The definitive antibiotic therapy must be guided by microbiological quantitative analysis with susceptibility test, in which the diagnosis of infection is granted by the presence of ≥ 10^5 CFU/g of deep and non-superficial (biopsy) tissue of the wound. The time of the antibiotic administration must be the least necessary to control the symptoms (one to two weeks for soft tissues infection and six weeks for osteomyelitis)[46]. If performed material sampling through swab, use the Levine technique[2]. Avoid using topic antibiotics, for this type of drug is not appropriate for treating polymicrobial flora, once it is difficult to adjust its concentration, as well as because of the risk of inducing antibiotic resistance[2].	WUWHS-Bjarnsholt, 2016[5] Snyder, 2017[40] Schultz, 2017[4] Lipsky, 2016[46] IWII-Swanson, 2016[2]
9. Use products with technology able to tear the polymeric extracellular matrix, which structures and protects the microorganisms present in the biofilm, as well as to break the link among said organisms and the matrix and/or to interrupt communication among several microorganisms, exposing them so that an effective microbicide treatment can be applied[40,47]. Example: PHMB, benzethonium chloride gel (high osmolality surfactant)[40], hypochlorite, cadexomeer iodine, silver hydrofiber , EDTA and benzethonium cholide[48].	Snyder, 2017[40] Wolcott, 2015[47] Parsons, 2016[48]
10. Treat topically and systematically the chronic infection of the wound.	WUWHS-Bjarnsholt, 2016[5]
11. Improve patient's immunologic competency.	WUWHS-Bjarnsholt, 2016[5]

D. Antimicrobial and antibiofilm agents

The discussed document states that there are treatment and prevention strategies, including debridement and the use of drugs to eliminate biofilm, but none of the named strategies is considered actually effective, making that the primary care must focus on reducing microbial load and preventing biofilm formation. Consequently, although appropriate to the recommendation, the content is not sufficiently comprehensive, lacking a more profound discussion about all the products available in the Brazilian market, as well as a better elucidation of information.

International consensus and guidelines for the clinical practice conclude that biofilm treatment is not possible after a single intervention or product, but through a group of interventions, including periodic evaluation[4,5,40]. Several substances and treatments, available or not in Brazil, belong to a long list of antibiofilm agents, which must be better discussed. They are listed in Table 2.

FINAL CONSIDERATION

Mercury organic compounds, alone, are considered obsolete antiseptic[3]. There were not found studies that

support the use of cranberry and N-acetylcystein (NAC) for preventing or controlling biofilm in wounds. Hydrogen peroxide, or oxygenated water (H_2O_2), is considered obsolete for use in wounds, once, in concentrations as of 8.5 mg/l, it already inhibits the proliferation of fibroblasts, while bacteria continue to be viable[3,56]. It is worthy to highlight that H_2O_2 is formed in concentrations non-cytotoxic in medical honey by the reaction of glucose oxidase, but this effect does not compare to external H_2O_2, when applied alone and pure[3].

Table 2. List of scientific evidences of antibiofilm action in products and treatments for wounds of difficult healing.

Antimicrobial drug	Source	Type of study	Synthesis of evidences against biofilm
Polyhexamethylene biguanide (PHMB)[3] Solution 0.1% and 0.2% Gel 0.1% Dressings 0.2%, 0.3% and 0.5% Available in Brazil	Kramer, 2018[3] EWMA-Gottrup, 2013[49] HSE-Nolan , 2018[43]	Consensus Systematic review Consensus	Action against biofilms caused by *E. coli, S. aureus*, and *P. aeruginosa*[49]. This product shows alkaline properties, attaches to phospholipids eisting in the bacterial cell wall and favors its destruction[43,49].
PHMB/betaine – Polyhexamethylene biguanide with betaine Gel and solution at 0.1% polyhexamethylene biguanide and 0.1% betaine Available in Brazil	WUWHS-Bjarnsholt, 2016[5] Kramer, 2018[3] IWII-Swanson, 2016[2] Bellingeri, 2016[50]	Consensus Randomized controlled trial	Betaine action (surfactant) prevents biofilm attachment to the lesion bed[2,50] and reduces superficial tension of the medium, helping in the cleaning process[2,3,5].
Acetic acid ($C_2H_4O_2$) – AA Solution and dressing at 0.25 to 2% Unavailable in Brazil	Bjarnsholt, 2015[51] Madhusudhan, 2016[52]	In vitro e in vivo Randomized controlled trial	In vitro: the concentration at 0.5% exterminated *P. aerugionsa* biofilm and reduced *S. aureus* bacterial load in biofilm; at 1% completely exterminated *S. Aureus* and *P. aeruginosa* biofilm. In vivo: the concentration at 1% associated to negative pressure therapy with instillation (NPTWi) was efficient against biofilms[51]. Dressings impregnated with acetic acid at 1% eliminated *P. aerugionsa* from chronic wounds[52]. The differentiated effect against biofilm is not yet clear.
Citric acid Solution and dressing at 3% Unavailable in Brazil	Malu, 2014[53] Watts, 2016[54]	In vivo Consensus	Action in wounds infected with *S. aureus, E. coli, Proteus* and *Klebsiella*[53] (level B evidence)[54]. However, there is a lack of studies that report its action against biofilms.
Sodium hypochlorite (NaOCl)/ hypochlorous acid (HOCl) Solution, spray and gel HOCl (0.04%) + NaOCl (0.06%)3 HOCl – 0.033%55 NaOCl (Dakin's solution) – 0.125%, 0.025%, 0.05%56, < 0,06%3 Available in Brazil	IWII-Swanson, 2016[2] Kramer, 2018[3] Day, 2017[55] Ueno, 2018[56]	Consensus Consensus In vitro and in vivo Review	Penetrates in biofilm causing its destruction[2,3]. Shows an action against methicillin resistant S. aureus (MRSA) and P. aeruginosa biofilms[55]. Shows bactericide activity for it is an oxidant agent[3,56] in concentration at 0.05% Currently, it is still employed for treating infected wounds in concentration at 0.125%. Depending on the concentration, it can be cytotoxic for fibroblasts[56].

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Table 2. Continuation...

Antimicrobial drug	Source	Type of study	Synthesis of evidences against biofilm
Octenidine dihydrochloride (OCT) Solution and gel at 0.05% Unavailable in Brazil	Wounds UK-Booth, 2013[57] IWII-Swanson, 2016[2] EWMA-Gottrup, 2013[49] Kramer, 2018[3]	Consensus Consensus Consensus Consensus	Acts managing biofilm in wounds[57], specifically in the inhibition of planktonic bacteria, as well as in bacterial biofilms for up to 72 h[2]. It is active against *P. aeruginosa* and *S. aureus* [49]. It has showed to be more effective against *P. aeruginosa* than ***S. aureus*** in comparison to other agents, but with reservations regarding the method and period of application[3].
Octenidine dihydrochloride / phenoxyethanol (OCT/PE) Solution and gel at 0.1% OCT and 2% FE Unavailable in Brazil	Kramer, 2018[3] Junka, 2014[58]	Consensus In vitro	It has showed to help removing biofilm when used as a gel, specifically in burning wounds. The solution, in its turn, can be used alongside with NPWTi[3]. Acts specifically on polysaccharides existing in bacterial cell wall, causing the leaking of cytoplasmic contents and compromising cell functions. It eradicated 100% of ***S.*** *aureus* and *P. aeruginosa* biofilm in 30 minutes of contact[58].
Chlorhexidine digluconate (CHD) Soap, solution and spray Concentrations: 0.12%, 0.2%, 0.5%, 1%, 2% and 4% Available in Brazil	Touzel, 2016[59]	In vitro	The solution at 0.12% was not able to penetrate the bacterial biofilm. Soaking the dressing and the gaze in CDH at 0.5% was effective in reducing *S. Aureus* biofilms in vitro, not showing effective outcomes against *P. aeruginosa, K. pneumoniae* and *E. faecalis.* This product's evidence for the treatment of chronic wounds is weak[59] and its use is considered obsolete[3].
Silver Powder, solution and dressings with several concentrations, such as: 25 µg/cm2, 1.2% ionic Ag, 60 ppm (particles per million) etc. Available in Brazil (powder and solution unavailable)	Percival, 2015[60] IWII-Swanson, 2016[2] Parsons, 2016[48]	Non-systematic review Consensus In vitro e in vivo	Shows to be effective against planktonic bacteria (free) in studies in vivo and in vitro[60]. Ionic silver and nanocrystalline silver at high concentrations display some efficiency against biofilm in models in vitro[60]. Low concentrations of ionic silver are effective for preventing new biofilm formation[2,60]. Ionic silver, alongside with surfactants (EDTA and BEC), hydrogels, fibrous materials and polyphosphates, shows antibiofilm potential[2,43,60].
Cadexomer iodine Dressing, ointment and powder Ointment 0.9% Available in Brazil	Wounds UK-Booth[57] WUWHS-Bjarnsholt, 2016[5] Kramer, 2018[3]	Consensus Consensus Consensus	It is active against MRSA and acts preventing biofilm formation[57]. Dressings with cadexomer iodine are showing to have action against planktonic bacteria and bacterial biofilms[3,5] of *S. aureus* and *P. aeureginosa*[3].

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Table 2. Continuation...

Antimicrobial drug	Source	Type of study	Synthesis of evidences against biofilm
Polyvinylpyrrolidone-iodine (PVPI) Water solution 1%, 7,5% and 10% Available in Brazil	Junka, 2014[58] Oliveira e Santos, 2008[61]	In vitro Systematic review	Solution at 7.5% in contact for 15 minutes eradicated 33% of *P. aeruginosa* and 100% of ***S. aureus***. On the other hand, 30 minutes of contact eradicated 66% of *P. aeruginosa* and 100% of *S. aureus*, both in biofilm[58]. A systematic review found that three of every five clinical trials showed favorable of its use for healing and prevention of infection, although with no proof being showed by metanalysis[61].
Ethacridine lactate Water solution 0.1% Unavailable in Brazil	Junka, 2014[58]	In vitro	The solution did not exterminated *P. aeruginosa* biofilm in 30 minutes of contact. However, it eradicated 100% of *S.aureus*[58] biofilm.
Proteolytic enzymes Gel, ointment and powder Concentration depending on pharmaceutical manipulation: 2 to10% Unavailable in Brazil	EWMA-Gottrup, 2013[49] Watters, 2016[62]	Consensus In vitro	Animal, vegetal or bacterial enzymes (papain, collagenase, streptokinase, bromelain and fibrinolysin) have a role in the debridement of non-viable tissue through peptide bonds hydrolize[49]. α-amilase, bromelain, lysostapin and papain showed effective in eradicating *S. aureus* biofilm, once they can reduce biofilm biomass, causing bacterial cellular damage due to the alteration of its morphology[62].
Dialkyl carbamoyl chloride (DACC) Impregnated dressing Available in Brazil	Totty, 2017[63] Wounds UK-Booth[57] WUWHS-Bjarnsholt, 2016[5]	Systematic review Consensus	It is showing promising outcomes in the treatment of infected wounds[63]. It is considered a passive antimicrobial with antibiofilm activity, since it attracts the microbial load from the lesion bed into the dressing[5,57].
EDTA (Ethylenediaminetetraacetic acid) Impregnated dressing Available in Brazil	Finnegan, 2015[64] WUWHS-Bjarnsholt, 2016[5] IWII-Swanson, 2016[2]	Review Consensus Consensus	Breaks biofilm EPS matrix, favoring topic antimicrobials action[5,44] and, combined with other antimicrobial contents, such as ionic silver, acts synergically to combat biofilm[2].
Medical honey Gel, ointment, dressings Quality classification by Unique Manuka Factor: 5 to 26 points65 Unavailable in Brazil	IWII-Swanson, 2016[2] Wounds UK- Booth[57] EWMA-Gottrup, 2013[49]	Consensus Consensus Consensus	Disrupts and prevents biofilm formation[2,57], and inhibits quorum sensing[2]. Prevents cell division in *Staphylococcus* and destroys cell membranes of *Pseudomonas*. It has antibiofilm activity to *P. aeruginosa, S. aureus* and MRSA[49]. It cannot be mistaken for common honey. The most used medical honey is Manuka's honey, for it is sterilized by gamma radiation and has strict quality control.

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Table 2. Continuation...

Antimicrobial drug	Source	Type of study	Synthesis of evidences against biofilm
Methylene blue (MB) and gentian violet (GV) Dressing of MB + GV Unavailable in Brazil	Edwards, 2014[66] Woo, 2014[67]	In vitro Review	Organic stains with potential to interfere in bacterial metabolism, specifically in the oxidation–reduction (redox) cycle, leading to its destruction. Nonetheless, their action in biofilms must still be proved[66]. They have a wide spectrum of action, including MRSA[67].
Lactoferrin e xylitol Hydrogel Unavailable in Brazil	Ammons, 2011[68] WUWHS-Bjarnsholt, 2016[5]	In vitro Consensus	Hydrogel containing lactoferrin and xylitol combined to a nanocrystalline silver dressing showed to be effective against MRSA and *P. aeruginosa*[68] biofilms. It has antibiofilm action, once lactoferrin adhere to the cell wall, causing its destabilization and death, and xylitol interferes in bacterial metabolism, leading to their distruction[5].
Gallium Iron-chelating deferiprone (Def) and heme analog gallium-protoporphyrin (GaPP) Unavailable in Brazil	Richter, 2017[69] WUWHS-Bjarnsholt, 2016[5]	In vitro Consensus	Def acts as an iron-chelating on bacterial membrane, and GaPP acts as an analog to iron. It were tested[69]: 1) Gel containing Def; 2) Gel containing GaPP. Both showed to have action against *Staphylococcus* biofilm; 3) The combination of Def with GaPP was effective against *P. aeruginosa* biofilm; 4) The combination of Def, GaPP and ciprofloxacin was effective against different multidrug-resistant (MDR) strains. Gallium is showing positive outcomes in preventing biofilm[5].
Bacteriophage/Phage Phage therapy Unavailable in Brazil	Flores, 2010[70] Rhoads, 2009[71]	Non-systematic review Experimental study in vivo	Viruses that infect only bacteria and act as natural predators. They have the power to penetrate biofilms inducing the production of enzymes that degrade the matrix of EPS matrix[70]. Study about phase 1 reported that a specific bacteriophage is safe against *P. aeruginosa, S. aureus* and *E. coli* in patients with venous leg ulcer[71].
Essential oils (EO) Oil solutions Available in Brazil	García-Salinas, 2018[72] Sharifi, 2018[73]	In vitro In vitro	Break planktonic bacterial membrane. The combination of EO compounds in concentrations higher than 0.5 mg/ml prevented biofilm formation and eliminated pre-formed *S. aureus*[72] biofilm. Positive outcome in preventing biofilms, as well as eliminating ***S.aureus***[73] biofilms.

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Table 2. Continuation...

Antimicrobial drug	Source	Type of study	Synthesis of evidences against biofilm
Amino acid (tryptophan) Unavailable in Brazil	Courrol, 2019[74] Brandenburg, 2013[75]	In vitro In vitro	Study in vitro tested silver and tryptophan nanoparticle (TrpAgNP). This compound infiltrates on the bacterial cell wall in planktonic state, as well as in the biofilm EPS matrix, causing damage and cellular death. The substance showed to be effective against *S. aureus* and *S. epidermidis*, as well as against *E. coli*[74] biofilms. It increased bacterial flagellar motion, inducing their detachment from biofilm, and showed effective outcomes against *P. aeruginosa*[75] biofilm.
Rotary magnetic field (RMF) System Available in Brazil	Junka, 2018[76] Bandara, 2015[77]	In vitro In vitro	RMF (10 to 50 Hz), alongside with the use of antimicrobials, reduced in 50% *S. aureus* and *P. aeruginosa* biofilm formation and mass, displaying adjuvant qualities for treating wounds[76]. It shows significant results in destroying *P. aeruginosa*[77] biofilm and preventing its formation
Negative pressure wound therapy System Unavailable in Brazil	Kramer, 2017[3] Kim, 2013[78] Tahir et al., 2018[79] ESCMID Biofilm Guideline- Høiby et al., 2015[80]	Consensus Consensus In vitro Consensus	It may contribute in biofilm removal when used for instillation with or without antiseptic, immersed in the wound bed for a given period of time, draining the fluid posteriorly during the activation of negative pressure[3,80]. Degree of recommendation CIII by ESCMID[80]. The activity against biofilm depends on the use of antiseptic solutions[78]. Instillation per se alters only the architecture of biofilm, reducing its thickness and mass, not affecting, however, bacterial cellular viability against *P. aeruginosa* and *S. aureus*[79].
Ultrasound treatment System Available in Brazil	Seth, 2013[81] Rastogi, 2019[82] EWMA-Strohal, 2013[44] HSE-Nolan, 2018[43] Murphy et al., 2018[83]	In vivo - animal model Randomized Controlled Trial Clinical practice guideline Consensus Consensus Randomized Controlled Trial	The action mechanism upon biofilm is not entirely elucidated. Nevertheless, reduction of the bacterial load[81,82] and of EPS matrix of *P. aeruginosa*[81] was observed. It produces microbubbles on the wound surface, which detach the biofilm from the lesion bed[44]. Safe method, that can be employed as an adjuvant for treating chronic wounds with evidence degree A of HSE recommendation[43]. It is suggested that it increases the susceptibility of the biofilm to the penetration of the antimicrobials, stimulating the increase of its metabolism[83].
Ozone therapy System Available in Brazil	Fitzpatrick, 2018[84]	Systematic review	Oxidizes lipoproteins and phospholipids existing in the bacterial wall membranes, causing irreversible damage. The action of ozone therapy is yet to be elucidated.

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Table 2. Continuation...

Antimicrobial drug	Source	Type of study	Synthesis of evidences against biofilm
Larval therapy Sterilized *Lucilia sericata larvae* Available in Brazil	EWMA-Strohal, 2013[44]	Consensus	Acts against biofilm, once it causes debridement in the devitalized tissue of the lesion bed. Acts specially against Gram-negative species of bacteria, primarily *E. coli*[44].
Hydrosurgery System Available in Brazil	EWMA-Strohal, 2013[44]	Consensus	Intense and fast irrigation of the wound is able to remove non-viable tissues and biofilm. What is more, it can be considered an enhancer of the action of antiseptic solutions when used together[44].

CONCLUSIONS

It can be concluded that the "1st Brazilian Recommendation for Biofilm Management in Chronic and Complex Wounds" is the result of an initiative to satisfy the current demand, in Brazil, to adapt to international recommendations for managing infected wounds with suspected biofilm. However, it is no advised to use it as a guide of clinical recommendations, since it deeply needs review, as well as methodological and bibliographic adjustment. Hence, it is suggested the translation and validation of an algorithm for managing biofilm, following the most recent international consensus, in order to help the clinical practitioner's decision making.

AUTHOR'S CONTRIBUTIONS

Idealization, Santos VLCG; Initial critical evaluation, Beteloto-Silva O, Coelho MF, Queiroz WMS and González CVS; Methodology, González CVS; Literature review – First wording, Beteloto-Silva O, de Souza DMST, Coelho MF, Queiroz WMS, Thum M and González CVS; Writing – Proofreading and Edition, González CVS, Thum M; Ramalho AO; Beteloto-Silva O and Coelho MF; Supervision, Nogueira PC; de Souza DMST and Santos VLCG.

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