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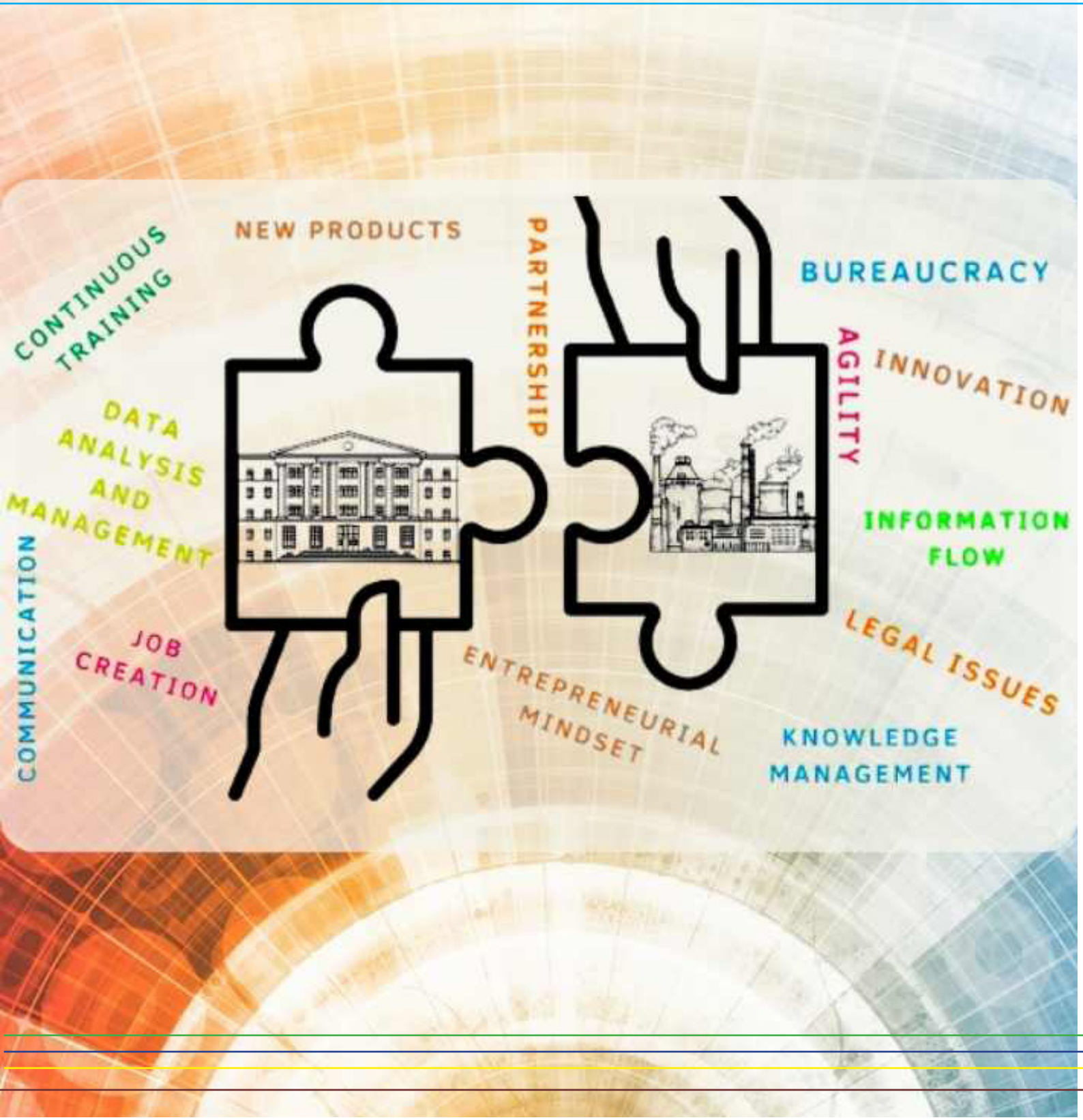

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Innovation projects at the University–Pharmaceutical Industry interface: challenges and opportunities

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Abstract: COVID-19 pandemic accelerated the digital transformation of pharmaceutical R&D. This paper aimed to diagnose some key aspects of University–Pharmaceutical Industry (U–Pharma) partnerships in Brazil that should be addressed in digital platforms to enable the creation of innovative solutions to COVID-19. A qualitative research was applied through an online guiding questionnaire to identify the main points that are considered as opportunities or challenges by the parties involved in the University–Pharmaceutical Industry interface. The responses were processed using content analysis to raise common themes. Further, in-depth interviews were performed to evaluate the subjective perception of these themes. The results of the questionnaire showed the topics bureaucracy, partnership, and agility as the most relevant. The in-depth interviews showed the subjective perception of these themes. The joint analysis of the results showed that in addition to problems related to project management processes, issues that involve the human dimension, such as pro-executive behavior and communication skills, are also extremely relevant to the success of U–Pharma projects. The development of digital solutions for Knowledge Management of innovative projects of U–Pharma collaborations should take into consideration both the project and human dimensions. It is recommended that Project and People Management should be integrated into digital platforms.

Keywords: University–Pharma Industry Collaboration. Digital Solutions. Project and People Management. Pharmaceutical Innovation.

Introduction

The COVID-19 pandemic has considerably changed the work organization of the population worldwide [1]. The articulation of the University–Industry interface in innovative projects that aim to meet the urgent demands imposed by the crisis is of fundamental importance. Chemical and Pharmaceutical Research is one of the most important representative interactions that occur at the University–Industry interface. The so-called University–Pharmaceutical Industry (U–Pharma) collaboration has a great potential to develop innovative solutions to deal with the pandemic scenario, whether in terms of prevention, treatment, or diagnosis [2].

Although cooperative projects have been taking place for a long time, the full development of this interface still seems to be quite challenging, even in developed countries [3,4]. Because University and Industry have fundamentally different goals and are formed by researchers with different profiles, aligning strategic actions on both sides of the coin often run into problems that are difficult to resolve. Also, the current crisis requires increased attention by the bodies responsible for applied research to develop strategically, considering strong connections with fundamental research [5,6]. The areas of communication and project management are identified as important points of attention that directly interfere in the success or failure of these partnerships [7,8].

The so-called digital revolution, which has been present for decades with the creation of the internet, had its pace accelerated with the new reality imposed by the COVID-19 pandemic [9,10]. On the University side, the educational and work processes are being profoundly modified [11]. Most of the undergraduate and graduate courses are being transformed to meet the current scenario. Didactic and research activities are being changed to the online format, which is increasingly requiring teachers and students to adapt.

The Industry side is also rapidly changing as it consolidates the insertion of Industry 4.0 and the entire arsenal of concepts and new technologies related to information systems (eg "Big Data", "Machine Learning", Artificial Intelligence, among others). Particularly, the COVID-19 pandemic and the Industry 4.0 seems to influence each other. COVID-19 perturbed the supply chain outcomes (e.g. sales and market share losses, delivery delays, declines in service and customer satisfaction. At the same time, Big Data analytics have been pointed as a useful solution to improve supply chain resilience in comparison with other information technology tools [12]. The Pharmaceutical Industries, for example, have been showing great interest and capacity to absorb these new tools seeking to improve productivity

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and processes [13].

Concerning the University–Industry interface, digital transformation can contribute significantly through the automation and facilitation of processes that demand excessive time on the part of the members that compose the interface. The natural result of this is an improvement in the processes that make up the management of projects that occur in collaboration and, mainly, an advance in knowledge management by gaining agility and security in the flow of data and information that support the decision–making processes

The objective of this work is to establish a diagnosis of the main challenges and potential opportunities within innovation projects that occur at the U–Pharma interface. The following guiding questions were used for the development of the research:

Q1 – How is the U–Pharma interface in Brazil characterized at the moment of the COVID–19 pandemic?

Q2 – What is the perception about the challenges and opportunities of the parties involved in the Pharmaceutical Science and Innovation development loop?

Methods

In this paper, exploratory qualitative research was used to establish a diagnosis of the current situation of the U–Pharma interface. As it is a complex social nucleus and composed of individuals with very different profiles, it was decided to use a multimethod approach using quantitative and qualitative data. The combination of these two approaches is important to establish more clearly the objective and subjective points experienced by the actors that make up the collaborative interface [14]. Online questionnaire was used to explore the general points by means of quantitative data. In–depth interviews were used to focus on the subjective aspects of the points raised in the online questionnaires [15].

Questionnaire design

The online questionnaire was the first step in the work aimed at the parties involved in the innovation projects that take place at the U–Pharma interface: i. researchers inserted in the University (academics), ii. researchers in the Pharmaceutical Industry (raw material and/or finished product) and iii. Startup researchers working on this interface. The questionnaire was prepared on the "Google Forms" platform and disseminated on the internet via "e–mail" and social networks ("Linkedin" and "Facebook"). The questions were organized into three information axes: Axis 1 – Characterization of the personal and professional profiles of the stakeholders that make up the interface, Axis 2 – Identification of Challenges and Opportunities that occur in the collaborative interface and 3 – Evaluation of the perception of value about products and services resulted from collaborative projects.

Some open questions were also inserted in the online questionnaire to identify relevant topics to be dealt with in more direct interaction with each of the parties involved. The content analysis of the answers to these questions followed the methodology proposed by Laurence Bardin [16]. The coding of the responses was done by defining units of semantic and context registration using the qualitative analysis software ATLAS.ti. The themes emerging from this codification were then classified into the Challenges and Opportunities categories and were used as a basis for the construction of the in–depth interview script.

Interview design

The in–depth interviews were designed to clarify the subjective aspects inserted in the information axes 2 and 3 of the research. More specifically, it sought to understand how each of the parties involved perceives the situations of challenges and opportunities that are experienced in U–Pharma partnerships. A semi–structured questionnaire was used for this purpose. This stage of the work was aimed at participants previously selected from the responses to the online questionnaire. The interviews were recorded with the interviewees' permission and then later analyzed using the ATLAS.ti software. The online questionnaire and interview guide can be found in Supplementary Information.

Ethical issues

The information provided by the online questionnaire was treated anonymously to ensure confidentiality. The participant's responses were reported and analyzed using numerical codes to ensure that data cannot be linked to a specific individual. The interviews were conducted with the consent of the participants. All participants were aware of the anonymity and objectives of the research.

Results and Discussion

The U–Pharma interface has a great potential to generate innovations to deal with the current situation of the COVID–19 pandemic. However, it is necessary to recognize the perceptions of the main parties involved about the evolution of partnership projects. The results presented in this work seek to clarify how objective and subjective factors may be positively or negatively impacting the outputs of collaborative projects. These results were understood through a careful analysis of the perception of the parties involved. A total of 40 respondents were counted. These respondents comprise the three main nuclei involved in collaborative U–Pharma projects: i. University, ii. Pharmaceutical Industry and iii. "Startups".

Characterization of the stakeholders that make up the University–Industry interface

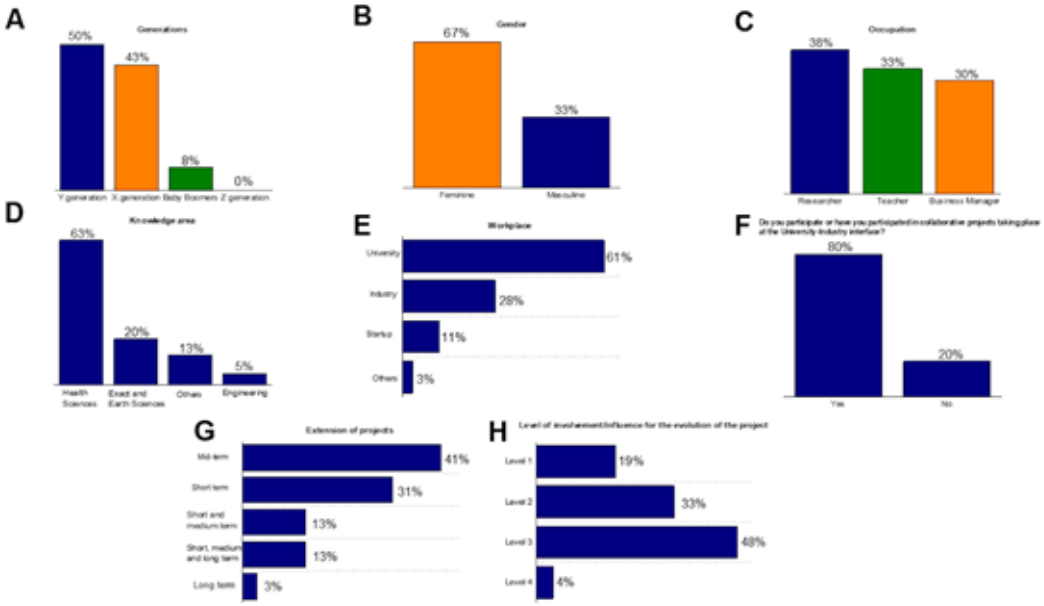
The socio–demographic characterization articulating the personal and professional profiles of the respondents

aims to raise "insights" about possible relationships between the perceptions of challenges and opportunities with characteristics such as sex, age, professional training, position, and level of influence in decision–making processes important for the evolution in collaborative projects of the U–Pharma interface. The characterization of the respondents' profile, presented in Fig. 1A–B, showed that most of them are female (67%) and are in the age group of 20 – 39 years (50%), belonging to the so–called Generation Y. Other participants are in the age group of 40 – 55 years (43%) and 56 – 74 years (7%), being classified within Generation X and "Baby Boomers", respectively. No respondent was representing Generation Z, which includes people under the age of 20. This classification of respondents following a generational approach is based on recent research that points out how the age group of workers can interfere with their perceptions and values [17].

The characterization of the professional profile is shown in Fig. 1C–H. Respondents have positions as business managers (30%), researchers (38%), and teachers (32%). Most respondents (63%) were classified as having training in the area of Health Sciences, being mainly pharmacists. Next are professionals with training in Exact and Earth Sciences (20%), formed mainly by chemists. Concerning the workplace, 61% of respondents are affiliated with a University, 28% with Industry, and 11% with a "startup" that interfaces between the academic and business sectors.

Most respondents (80%) have already participated in collaborative University–Pharmaceutical Industry projects. Most of these projects (41%) are medium–term, with a development period of 1–5 years, followed by short–term projects (31%), with a development period within one year. Long–term projects (over 5 years) total only 3%. This result may reflect a disarticulation of the University and Industry in disruptive innovation projects, which in general are long–term and involve the entire chain that unifies basic and applied research, from discovery, invention to innovation in the market. Regarding the level of influence on the decision–making processes that guarantee the evolution of the project, most respondents have some type of participation, directly (33%) or indirectly (48%). A still significant minority (19%) of the respondents is responsible as the sole decision–maker. This result can be evidence of the horizontalization of project dynamics within organizations [16]

Figure 1– Panels (A–B) Characterization of the respondents' profile. Panels (C–H) Characterization of the respondents' professional profile. Extension of projects defined according to the duration, being short (up to 1 year), medium (1–5 years), and long (over 5 years). For panel H, consider the following levels of participation in the decision–making processes: Level 1 (total participation), Level 2 (some direct participation), Level 3 (some indirect participation), and Level 4 (no participation)



Identification of challenges and opportunities from the point of view of different stakeholders

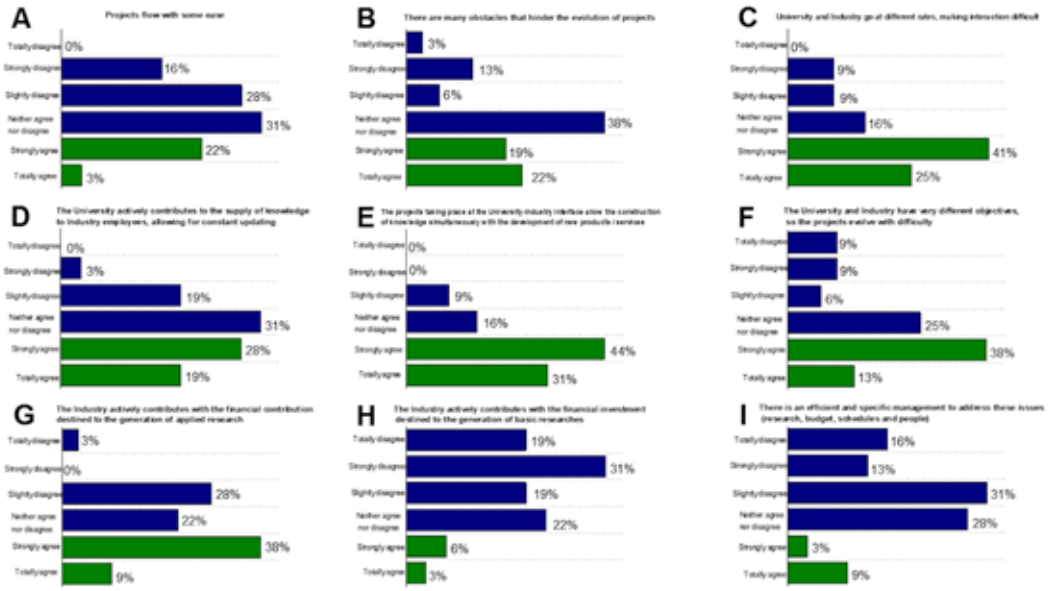
Challenges and opportunities are always realities that go together within an organization and can lead to the success or failure of projects. The differentiation between problem and opportunity must always be analyzed at an individual and collective level and, whenever possible, must be confronted with the potential risks for the evolution or stagnation of the project [19,20]. Fig. 2 presents the perspective of the different respondents about the perceptions of possible challenges and opportunities that occur during the development of collaborative projects. The results show that there are difficulties in the development of projects occurring in the collaborative interface between the University and the Pharmaceutical Industry, which can be demonstrated by numbers such as only 25% agree in some way that the projects flow easily and 41% agree in some way on the existence of obstacles (Fig. 2 AB).

The difficulties encountered in the evolution of such projects can be partly explained by the different mental models that comprise the parties involved belonging to the academic and business environment. The differences in work rhythm and purposes are shown in Fig. 2–C and F, which shows that 66% of respondents agree in some way about the University and Industry moving at different paces and 38% agree very much that they have very different objectives. Such factors are potential problems that hinder productive interaction during the development of collaborative projects and can be partially explained by the lack of active management at the interface (Fig. 2 – I). Here the results show that about 60% of the respondents disagree in some way that there is efficient and specific management to deal with research, budget, schedule, and people involved in collaborative projects.

Recognizing opportunities amidst countless challenges is not always easy, but it is essential, especially in times of crisis such as this that was triggered by the COVID–19 pandemic. In any partnership, opportunities, as well as challenges, need to be managed together. Fig. 2 establishes some points that can be seen as valuable opportunities in the U–Pharma collaborative process. The University is a nucleus of knowledge generation and, therefore, has the potential to offer an environment conducive to the development of projects that generate discoveries and inventions. However, this potential does not seem to be expressed in the current reality. When respondents were asked about the University to actively contribute to the knowledge input for the constant updating of Industry employees, about 22% disagreed in some way and 31% neither agreed nor disagreed (Fig. 2–D).

Possible discrepancies in the respondents' perception of the University's contribution to industry projects were also recorded. Some 75% of respondents agree in some way that University–Industry interface projects allow the construction of knowledge simultaneously with the development of new products (Fig. 2–E). The Industry's contribution to the research carried out at the University, especially applied research, divides opinions: 22% do not agree or disagree, 28% disagree a little, and 38% strongly agree (Fig. 2–G). On the other hand, about 69% of respondents disagree in some way that Industry contributes to basic research (Fig. 1–H). These results may show that the Industry has no interest in investing in this category of research since these are projects that aim to answer more fundamental scientific questions and, possibly, have a low potential to become a product in a short time.

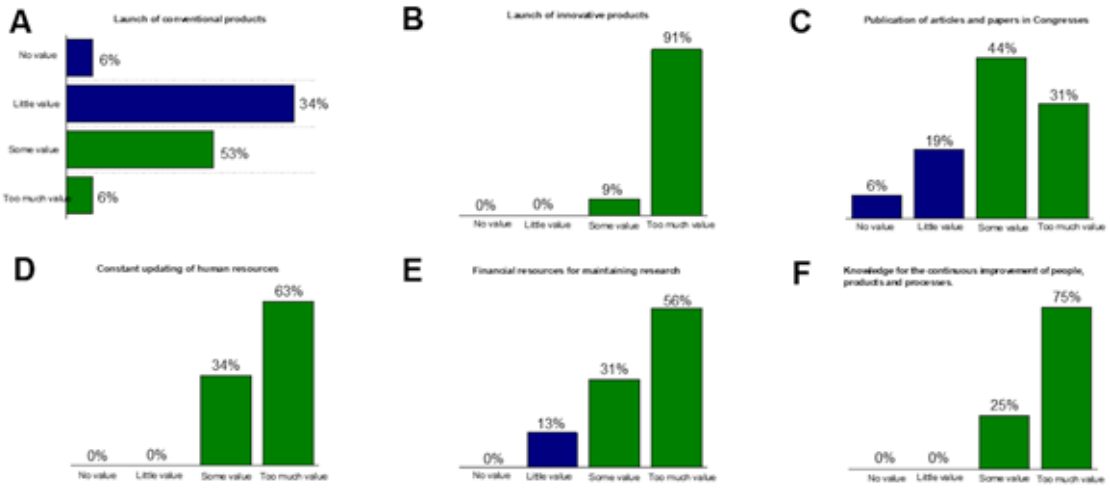
Figure 2– Diagnosis of the main topics that constitute challenges and/or opportunities in collaborative projects University – Pharmaceutical Industry. The statements were rated on a five–point scale: Totally agree – Strongly agree – Neither agree nor disagree – Strongly disagree – Totally disagree



Evaluation of the perceived value by the respondents about the collaborative interface outputs

The perceived value is a strategic element in any type of collaborative relationship. Needs and expectations must be negotiated between partners for effective collaboration. In the context of U–Pharma collaborations, we can analyze these values by analyzing the link between the generation and transfer of knowledge by the academic environment and the launch of products by Industry. Fig. 3 presents the respondents' opinion of some of the main outputs of the collaborative relationships that occur in the U–Pharma interface. Most respondents attach great value to the launch of innovative products (91%) while conventional products have some value (53%) (Fig. 3 – A and B). Regarding the publication of articles and papers in congresses, only 31% consider it to be a very valuable item (Fig. 3 – C). On the other hand, the majority of respondents attach great importance to the constant updating of human resources (63%), financial resources for maintaining research (56%), and knowledge for the continuous improvement of products, people, and processes (75%) (Fig. 3 – D, E, F).

Figure 3– Evaluation of perceived value by respondents in collaborative projects at University – Pharmaceutical Industry interface



Open questions

The open–ended questions at the end of the online questionnaire aimed to raise the main topics that respondents perceive as challenges or opportunities within collaborative U–Pharma projects. The emerging themes were coded from the responses and are shown within two categories (Challenges or Opportunities) in Table 1. The Challenges category accounted for seven emerging themes, of which "bureaucracy", "partnership", "agility" and "communication" were the most frequently cited: 12, 11, and 7 citations, respectively. The Opportunities category counted 9 emerging themes.

The issues within this category sought to cover mainly opportunities that can be used within Digital Platforms, which focuses on solutions based on Information Technology. The thematic codes "streamline the flow of information", "increase the efficiency of knowledge management" and "improve data analysis and management" were the most cited: 22, 16, and 16 citations, respectively. All of these emerging themes within Challenges and Opportunities comprise important dimensions of Project Management. Although it is not conclusive, these results may show the lack of adoption of some management techniques and good practices that can assist in the conduct of collaborative projects aimed at mitigating problems and maximizing opportunities.

In–depth interviews

In–depth interviews were conducted to improve understanding of emerging issues in the open–ended questions in the online questionnaire. Within this context, the more subjective factors that involve the perception of what constitutes a problem and a challenge and what an opportunity could be better understood through direct interaction with three interviewees, each representative of a nucleus participating in collaborative U–Pharma projects. The main excerpts of the interviews conducted are shown in Table 2 within each of the themes.

Table 1 – Codification of emerging themes in the open questions of the online questionnaire

Identification of Challenges and Opportunities	Thematic Codes	Frequency
Challenges	Communication	4
	Partnership	11
	Beaurocracy	12
	Agility	7
	Management	1
	Misaligned goals	3
	Legal issues	1
Opportunities	Knowledge generation	4
	Development of new products	4
	Innovation	2
	Entrepreneurial mindset	1
	Job creation	1
	Streamline the information flow	22
	Increase the efficiency of knowledge management	16
	Increase the efficiency of employee trai–ning	10
	Improve data analysis and management	16

Table 2 – In–depth interviews of respondents worki

Guiding themes	***Interviewee 1 (Startup researcher working at the U–Pharma interface)***
Partnership	Partnership is something both sides benefit from; and the gains are not necessarily financial; Industry can benefit from access to equipment and analyzes carried out in University laboratories; University can benefit from putting interns in direct contact with Industry. Difficulties in executing projects that connect multiple areas; Academics lack a corporate vision; Need to develop behavioral skills rather than techniques
Agility	The industry is driven by profit; The academy has no incentive to move more quickly; The academy's pace is slower and the industry is faster; Agility is related to the extension of the project; Startups are also accelerated, but those that develop materials aimed at Health have a slower pace; Industries have processes that streamline work; University–Pharmaceutical Industry Interface can benefit from hybrid management models; Academics need to develop knowledge about management and planning models.

ng at the University – Pharmaceutical Industry interface.

Main perceptions on emerging themes	
Interviewee 2 (Academic researcher)	***Interviewee 3 (Industry rsearcher)***
Partnership happens when two people make an agreement to generate mutual benefit; The benefits need to be clear and are not necessarily financial; Gains related to knowledge and training of people serve as motivation. The "Job rotation" between researchers from University and Industry can be a healthy way to develop partnerships.	Partnership is risk sharing; For Industry, partnership is a business and for academics it is a funding opportunity; Partnerships happen when the Industry sees the potential to generate a product; Partnerships can help academic researchers advance their research towards new product launches. The focus of Industry and University are different; University must explore early–stage and proof–of–concepts while Industry must focus on manufacturing;
In short–term projects, agility is dependent on the level of knowledge of the research group on the subject in question; If the group needs to develop new knowledge, this can impact on agility. It is important to set expectations on both sides based on the objective, extent of the project, and the expertise of the academic research group on the specific problem that must be resolved.	Industry prefers to pay more for a university abroad; University needs to be more executive and know how to deal with task distribution and data management; The transfer of data from the University–Industry occurs in a disorganized manner; Industry values raw data; Pharmaceutical Industry is very regulated and is aligned with Anvisa. Hybrid models are suitable for interface projects: part of the regulation must be procedural and the development of ideas can be agile.

►►

Table 2 – In–depth interviews of respondents working at the Univ

Communication	Too many meetings can cast off the partnership; The nature of the subject to be dealt with should dictate the best communication approach.
Beaurocracy	Less bureaucratic alignments; Some institutions are already resolved; Need for people to resolve legal and intellectual property issues.
Digital Transformation	Too many online meetings can hurt; Assist in improving communication; Need for digital education;
Conclusive points about Challenges and Opportunities	The opportunities are still very idealized; Bureaucratic and behavioral skills issues are the main barriers; Brazilian collaborative management model needs to be built; Complete horizontalization of working relationships is not ideal. Some degree of hierarchical structure is still needed; There is an excess of theoretical information on entrepreneurship. Practical training is required.

versity – Pharmaceutical Industry interface (to be continued)

Openness is needed to understand what the partner wants. Industry problems sometimes arrive indefinitely or are very widespread. If knowledge management is not done, communication errors can persist throughout the development of new products.	Communication must be clear and the experimental steps pre–defined; There is a lot of difficulty for academic researchers in transforming experimental tests into deliverables; There is a lack of knowledge about the fundamentals of management / communication at the University; There is a need to build study groups at the University with a focus on developing teachers and students in relation to management / communication with the executive world; Lectures on entrepreneurship should be less vague and more objective.
Intellectual property is the most sensitive issue to be discussed; Innovation offices must be agile and respond quickly to these questions;	Intellectual property is the most sensitive issue to be discussed; Contract establishment is very bureaucratic, which impacts on agility. There is confusion about understanding the difference between service delivery and partnerships. Royalty sharing is a sensitive topic and needs to be treated with caution. The Industry has the know–how that can direct the project to the most appropriate way to generate a new product. The Technological Innovation Centers (NIT) deal with legal issues, but the level of preparation depends on the University.
Information Technology can facilitate University–Industry interaction through digital training; Experimental issues must be resolved in person. Computerization/ Automation of laboratory equipment.	The digital medium was already widely used by industry in the pre–pandemic period;
Challenges and Opportunities must be analyzed on a case–by–case basis; There are many academic demands upon the teacher that hinder the process.	The ideas that academic researchers propose for Industry are very vague; Challenges and opportunities coexist; There are many problems, but there are also many success stories; Product ideas are very difficult. In addition to the idea, it is necessary to have a promising market; The most difficult thing is to have the innovative idea.

The guiding theme "partnership", which had 11 citations in the online questionnaire, has the formal meaning of meeting people by common interest or objective, company, society. The exploration of this theme in in–depth interviews allowed us to identify that, in the interviewees' view, its most tangible meaning comes close to a division of gains and risks. Here, the highlighted gains and risks are not necessarily financial, but they must be very clear among stakeholders from the beginning. Besides, such gains and risks are different for the interviewed parties. While the academic side sees as gaining knowledge generation and training people, the Industry side needs to see the potential of research to become a product. This result is in line with what had already been exposed in the online questionnaires, where it became evident that the Industry's greatest interest in collaborative projects is centered on applied research.

Some other factors were also mentioned within the discussion of the partnership theme, as a way to understand why it sometimes becomes unviable. The lack of corporate vision and executive profile of academics was mentioned by two of the interviewees. As researchers located on the academic side do not always have this type of training, they may lack the most appropriate approach to deal with business issues, which makes it difficult to establish partnerships. Also, one of the interviewees highlighted the difficulties in establishing partnerships in projects that connect multiple areas. Such projects need to go beyond the technical–scientific knowledge of the people who make up each area and need to be developed mainly around solid behavioral skills.

The guiding theme "agility", which had 7 citations in the online questionnaire, has as its main meaning the speed of movement. The agile paradigm emerged in the 1990s in response to more traditional project management methods, which are heavier (eg "waterfall" method). Through the interviews, it was evident that all respondents perceive University and Industry walking at different paces. The industry is moving fast, driven by profit and the University is moving at a slower pace, driven by knowledge. This was also evidenced in the online questionnaires. However, it was also shown here that startups that operate at the University–Industry interface, despite being admittedly more accelerated, this will depend on the area of operation. The area of Health and Biotechnology is moving at a less accelerated pace when compared to the area of Information Technology, for example, since the first is subject to a series of steps and regulations. The regulation of the pharmaceutical industry by Anvisa, the Brazilian Government Agency of Pharma Regulation, was also mentioned by an interviewee as a way to clarify that not all departments are moving at an accelerated pace. Thus, the differences in rhythms occurring both internally and in the U–Pharma interface suggest that hybrid management models may be more appropriate than pure agile or traditional models.

The guiding theme "communication" had 4 citations in the online questionnaire and means "sharing", making it common, participating in something. It is through the act of communication that ideas can be shared and goals can be aligned. Respondents reported that although clear communication with pre–defined work steps is essential before development begins, this is not always possible. The interviewee on the academic side reported that the Industry side often brings ill–defined problems, which can hinder both communication and agility in proposing possible solutions. Besides, it was also highlighted once again the need for academics to develop a more executive posture to facilitate negotiations and the development of collaborative projects.

The guiding theme "bureaucracy", which had 12 citations in the online questionnaire, represents an organizational structure composed of rules, regulated processes, and an intense division of tasks and functions. Currently, the term has a negative connotation due to the intense value that is attributed to the number of rules instead of worrying about their meaning. The bureaucracy explored in the interviews was mainly related to legal issues involving the contractual and royalty division. This is considered to be the most sensitive topic and perhaps that is why it leads to delays in the process. Despite the existence of the Technological Innovation Centers (TICs), specific agencies to deal with these issues at Universities, not all of them have the same level of maturity that allows the accomplishment of tasks with the necessary agility. Also, it was evidenced by one of the interviewees that in many cases there is confusion about the nature of the projects that take place at the U–Pharma interface: partnership projects x service provision projects. These confusions can delay the progress of contracts since the question of "royalties" does not fit in the second category of projects.

The guiding theme "digital transformation", which encompassed codes referring to "streamlining the flow of information", "increasing the efficiency of knowledge management" and "improving data analysis and management" (total of 54 citations in the online questionnaire line), can be understood as a set of processes by which organizations seek to increase their performance both internally and externally. When analyzing the interviewees' perception on the topic, it became evident that the topic is more relevant mainly for the Industry side, which is very concerned with the issues of data agility and organization, since we are increasingly moving towards decisions based on evidence. Herein, automation, improved communication and the need for Digital Education were also raised on this theme.

Finally, the analysis of the conclusive points about the challenges and opportunities that occur in collaborative projects occurring at the University–Pharmaceutical Industry interface shows the need to decrease the level of idealization of the opportunities and move towards

more concrete management. It was also mentioned that the ideas must be treated less vaguely to facilitate the understanding of the parties involved. Furthermore, the need to train behavioral skills was again emphasized. These results are in line with the literature that explored the U–Pharma interface outside Brazil. As mentioned earlier, there is a strong perception among the main authors that the difficulties in taking advantage of opportunities are due to management and communication problems [21,22].

Answers to guiding research questions

Through the combined use of an online questionnaire with in–depth interviews, the following answers to the guiding questions of the research were obtained:

Q1 – How is the U–Pharma interface in Brazil characterized at the moment of the COVID–19 pandemic?

A. The characterization of the personal and professional profile: it has been shown that it is formed by people of different generations and different types of positions (teachers, researchers, and managers). Most of these people declared to have some degree of influence in the processes that involve the evolution of collaborative projects University–Pharmaceutical Industry, which can reflect the evolution of the work characteristics, being characterized by a progressive horizontalization of the relationships. This fact was also evidenced by one of the interviewees, although it was also emphasized that some hierarchical level must still be maintained in the relationships since the levels of maturity required for each type of decision are different.

Q2 – What is the perception about the challenges/opportunities of the parties involved in the Pharmaceutical Science and Innovation development loop?

A. The diagnosis of challenges and opportunities as well as the perception of the value assessed by the online questionnaire showed that these focuses are in both dimensions of processes and people. Concerning the challenges, the topics bureaucracy, partnership, and agility were the most frequently cited. The topic of bureaucracy was the most mentioned of all and was reported as one of the main obstacles to collaborative projects. Through in–depth interviews, it was realized that this theme is mainly related to legal and contractual issues in which specific points such as the nature of collaboration and the sharing of royalties can significantly delay or even make work unfeasible. The topic of partnership, the second most mentioned in the questionnaires, is perceived by the interviewees as a division of gains and risks. Agility, the third most cited, seems to be a highly dependent factor on the extent of the project and the level of knowledge around the demands of the project in question.

Conclusions and recommendations

The U–Pharma interface has been highlighted in the current times due to the demands generated by the COVID–19 pandemic. The need for innovative products and services capable of combating the pandemic is urgent, which makes it essential to better understand the main factors that dictate the interactions between the parties involved.

This work showed that the U–Pharma interface is creating increasingly complex social networks in which people of different ages and different mindsets must work together. In innovation projects, this is a reality for the group of respondents and interviewees. This mix of generations must be managed correctly so that natural conflicts can be translated into team learning. Through this research, it was realized that the focus of challenges and opportunities are not restricted only to the issues of processes that can be solved through information technology platforms, but also reach the dimension of people. Behavioral skills have been reported to be essential to the development of partnerships. These perceptions do not eliminate the importance of digital solutions, since one of the limitations of the research was the relatively low number of respondents (approximately 40 participants). Therefore, it is suggested that digital tools with a focus on improving project management processes also take into account the development and management of people. It is necessary to build digital businesses capable of connecting people and understanding issues involving human behavior is a central element to be considered. Future research in this line will be fundamental to deal with the new reality imposed by the COVID–19 pandemic. However, it will be necessary to evaluate a larger number of respondents to identify the perception of the groups that make up the U–Pharma interface at the national level and not only at the regional level.

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Bacteriological assessment of stethoscope used by healt care personnel in attat hospital, SNNP, Gurage Zone, Ethiopia

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Abstract: The stethoscope has always been an important element of a physician's toolkit when it comes to examining patients. The widespread use of stethoscopes by health-care workers for patient examinations makes them a potential source of nosocomial infection transmission. The goal of this study was to see if stethoscopes used by different health-care professionals in Attat hospital may transmit bacteria. From April to June 2018, a cross-sectional study was done in the molecular laboratory of Wolkite University's department of biotechnology and biology. A total of 26 stethoscopes from health workers who had direct contact with patients were gathered during the study period. The sample was obtained using a sterile cotton-tipped applicator saturated in a sterile solution of physiologic saline (0.85 % sodium chloride) to swab the whole surface of the stethoscope's diaphragm and then inoculated into macconkey agar, tryptone soya agar, and blood agar medium. 18(69.2%) stethoscopes out of total collected stethoscopes had bacterial growth and 12 bacterial isolates were selected and characterized to genus level. Isolates include staphylococcus aureus (37.5%), coagulase negative staphylococci (28.12%), Streptococcussp. (21.88%), and Bacillus sp.(12.5%). All isolates were susceptible to the co-trimoxazole and ciprofloxacine, while resistant to cifoxitine. They showed intermediate growth against vancomycine. All except streptococcus were found resistant against penicillin. Both S. aureus and CoNS were sensitive to the chloramphenicol; Streptococcus was intermediate while bacillus was resistant to the chloramphenicol. All stethoscopes (42.2 %) that had never been cleaned and were last cleaned a week ago were severely contaminated, while those washed multiple times a day and cleansed between each patient before the examination of the patients had lower levels of contamination (27 %).

Keywords: Bacterial Isolate. Nosocomial Infection. Stethoscope.

Introduction

Background of the study

Nosocomial infections have existed since the beginning of hospitals, and they continue to be a significant public health issue even in the modern era of antibiotics. When infections become clinically obvious during hospitalization (at least 72 hours after admission), they are classified as nosocomial[21]. Such infections are caused by a variety of factors, including the emergence and persistence of multidrug-resistant bacteria, patients' compromised immune systems, and mechanical transmission of microorganisms[12], all of which result in high morbidity and mortality, prolonged hospitalization, increased antibiotic use, and increased costs[12]. According to studies, these infections occurred in 5% to 10% of all hospitalizations in Europe and North America, and in more than 40% of hospitalizations in Asia, Latin America, and Sub-Saharan Africa[34].

According to[32], more than 1.4 million people globally are infected with illnesses acquired in hospitals at any given time, and health-care personnel are possible sources of these infections. Because many infections can be spread through the hands, all health-care professionals must wash their hands before and after each patient encounter[35]. Diseases can be transmitted through contaminated medical devices, and outbreaks of hospital-acquired infections have been connected to electronic thermometers, blood pressure cuffs, stethoscopes, latex gloves, masks, neckties, pens, badges, and lanyards, white coats, computers, and keyboards[31].

The sterilization and disinfection of intrusive equipment and devices prior to interventions are frequently overlooked. Stethoscopes are the most commonly utilized medical devices by health care personnel to examine the health of patients among those equipments. As a result, they frequently come into touch with a large number of patients and have been identified as potential nosocomial infection vectors in various regions of the world[26, 31, 27].

According to a similar report from Jimma University Specialized Hospital, bacterial contamination of the stethoscope is significant and could be a vector for illness transfer between patients and health care staff [29]. Pathogens can adhere and establish themselves on the diaphragms of stethoscopes after contact with contaminated skin, and then be conveyed to other patients if the stethoscope is not cleansed [18].

There are also more cases of antibiotic-resistant bacteria being transmitted from one patient to another via stethoscopes [31, 9, 20, 12]. In a hospital setting, these antibiotic-resistant organisms are capable of causing serious infections, necessitating contact isolation and rigorous treatment to limit the spread of the organisms[12]. Ceftazidime-resistant Klebsiella pneumonia, vancomycin-resistant enterococci, methicillin-resis-

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tant staphylococci, ciprofloxin–resistant Pseudomonas aeruginosa, gentamicin–resistant Pseudomonas aeruginosa, and penicillin–resistant pneumococci are examples of antibiotic–resistant organisms [16, 10, 22].

Infection transmission in hospitals (nosocomial infections) is a major issue caused by contaminated medical equipment and health–care workers (HCWs). Medical devices that have not been adequately sterilized/disinfected may spread bacteria from one patient to the next. Due to rising morbidity and cost burden, health–care–acquired infections are becoming a major concern not only for doctors, but also for patients, and stethoscope disinfection is still not a widely accepted practice among most health–care workers.

Despite the fact that stethoscopes are a possible vector for the transfer of health–care–associated illnesses and resistant bacteria, health–care professionals fail to disinfect them[31]. Swiping stethoscopes with alcohol pads is the current gold standard for stethoscope decontamination[27]. To prevent nosocomial infections, medical devices such as stethoscopes should be tested for microbial colonization on a regular basis, and health care staff should be educated on proper cleaning procedures [7].

Infection transmission in hospitals (nosocomial infections) is a major issue caused by contaminated medical equipment and health–care workers (HCWs). Medical devices that have not been adequately sterilized/disinfected may spread bacteria from one patient to the next. Because of rising morbidity and cost burden, health–care–acquired infections connected with stethoscopes are now a major concern for doctors as well as patients, and stethoscope disinfection is still not a widely accepted practice among most health–care workers. To our knowledge, there has only been one study on the function of stethoscopes in the transmission of nosocomial infections, and none has been conducted in Ethiopia's south. In Attat Hospital, a referral hospital serving people of ChehaWoreda and nearby communities in Southwest Ethiopia, we wanted to look into the role of stethoscopes as potential fomites for possibly dangerous bacteria. Therefore, this study was focus on the following objectives i) to establish the bacteriological agents responsible for stethoscope contamination in Attat Hospital, as well as to examine healthcare personnel's attitudes and knowledge about stethoscope hygiene behavior; ii) In order to identify and characterize bacterial isolates based on biochemical and morphological tests; iii) For determining a drug resistance profile of selective isolates; iv) To explore the behavior, attitudes and beliefs about stethoscope hygiene amongst healthcare personnel within the hospital's various clinical units.

Literature Review

The different types of nosocomial infections

The CDC and the National Healthcare Safety Network (NHSN) divide health–care–associated infection sites into 13 primary kinds based on clinical and biological criteria, with roughly 50 potentially specific infection sites for surveillance. Surgical wound and other soft tissue infections, urinary tract infections (UTI), respiratory infections, gastroenteritis, and meningitis are the most frequent nosocomial diseases that can arise in a hospital setting[25]. However, with the increased use of invasive procedures for therapeutic and diagnostic purposes, cancer chemotherapy, immunotherapy, and advancements in organ transplantation, changes in the distribution of nosocomial infection sites can be observed over time.

Epidemiology of nosocomial infections

It is estimated that about 10% of hospital patients or more than 2–million hospitalized patients are annually suffering from hospital infection in the USA; and an estimated annual death rate is 20,000, which may reach even up to 88,000 deaths per year. Basic epidemiologic patterns can be used to guide prevention and control actions in hospital–acquired infections. The virus that causes hospital infection has reservoirs, can be transferred in predictable ways, and needs a vulnerable host[33]. The inanimate environment, such as surgical instruments and the operating room, and the animate environment, such as diseased or colonized health care staff, patients, and hospital visitors, could be reservoirs and sources of infection. Cross–infection from an endogenous flora present in the patient or autoinfection from an endogenous flora found in the patient are two possible modes of transmission for hospital acquired infection. For example surgical site infection can be caused by an endogenous flora that translocate to a normally sterile site or when the sterile peritoneal cavity is contaminated by spillage from the gastrointestinal tract; and by an exogenous source of microbial contamination that comes from the surgical team, surgical instrument and the theatre environment.

Furthermore, aseptic procedures were not followed strictly by the majority of the nurses and physicians in several practice areas and are found to be significant for the transmission of the infection[24].

Nosocomial infections: sources and transmission

Infections are caused by nosocomial microorganisms that can come from either endogenous or external sources. Hospital staff, other patients, visitors, food, water, fomites, urinary catheters, intravenous devices, respiratory apparatus, and other prosthesis are all examples of animate and inanimate sources of exogenous infections. Contact is the most common way for nosocomial illnesses to spread, generally directly but occasionally indirectly through bodily secretions. Air can also be a source of airborne nosocomial viruses that infect the respiratory tract (e.g., in droplet nuclei and aerosols). Food–borne and water–borne diseases can

enter through the faeco–oral pathway. The oropharynx, gastrointestinal system, and urinary tract are the most prevalent reservoirs for nosocomial colonizers [26].

Nosocomial infection risk factors

For a variety of reasons, hospitalized patients are at an unusually high risk of infection. Intrinsic and extrinsic factors are roughly classified into two categories. Intrinsic risk factors are those that are present in the patient as a result of the underlying disease. Patient care may contain extrinsic risk factors. Concurrent infections, prosthetic devices, surgery, immunosuppressive medications, broad–spectrum antibiotic therapy, and the emergence of multidrug–resistant organisms are some of the general predisposing factors that make patients prone to nosocomial infections. Other risk factors include the patient's age, length of stay in the hospital, underlying conditions such as diabetes, malignancies, or ward congestion. The length of hospital stay is the most important risk factor for contracting a nosocomial infection among the multiple risk variables [17].

Nosocomial infection agents

Infections in hospitals are caused by a wide range of bacteria, and any bacterium has the potential to cause an infection in hospitalized patients.

Microorganisms

Nosocomial infections can be caused by a variety of microorganisms. The infecting organisms differ depending on the patient demographic, the health care setting, the facility, and the country.

A. Bacteria

These are the most commonly found nosocomial pathogens in hospitals. There is a distinction to be made between commensal bacteria found in the typical flora of healthy humans and pathogenic bacteria. These provide an important protective role by preventing harmful germs from colonizing the area. If the native host is harmed, some commensal bacteria may cause illness. Intravascular line infection is caused by cutaneous coagulase negative staphylococci, and urinary infection is caused by intestinal Escherichia coli.

Pathogenic bacteria have a higher pathogenicity and, independent of host status, cause infections (sporadic or epidemic). Anaerobic Gram–positive rods (such as Clostridium) induce gangrene, for example.

Gram–positive bacteria: Staphylococcus aureus (a cutaneous bacterium that colonizes both hospital staff and patients' skin and nose) causes a wide range of lung, bone, heart, and bloodstream infections and is usually antibiotic–resistant; beta–haemolytic streptococci are also essential.

Gram–negative bacteria, such as E. coli, Proteus, Klebsiella, Enterobacter, and Serratiamarcescens, can colonize places where the host's defenses are impaired (catheter insertion, bladder catheter insertion, cannula insertion) and cause significant infections (surgical site, lung, bacteraemia, peritoneum infection). They could also be extremely resistant. Gram–negative bacteria, such as Pseudomonas spp., are frequently found in wet and damp environments. They may colonize hospitalized patients' gastrointestinal tracts.

Other germs provide a distinct threat in hospitals. For example, Legionella species can cause pneumonia (sporadic or endemic) in people who inhale polluted water aerosols (air conditioning, showers, and therapeutic aerosols).

B. Viruses

Many viruses, including hepatitis B and C (transfusions, dialysis, injections, and endoscopy), respiratory syncytial virus (RSV), rotavirus, and enteroviruses, can be transmitted nosocomially (transmitted by hand–to–mouth contact and via the faecal–oral route). Other viruses that can be transferred include CMV, HIV, Ebola, influenza viruses, herpes simplex virus, and varicella–zoster virus.

C. Parasites and fungi

Some parasites, such as Giardia lamblia, are easily spread between adults and children. Many fungi and parasites are opportunistic organisms that cause infections when the immune system is suppressed by antibiotics (Candida albicans, Aspergillus spp., Cryptococcus neoformans, Cryptosporidium). In immune–compromised patients, they are a primary source of systemic infections. Contamination of the environment by airborne organisms that originate in dust and dirt, such as Aspergillus spp., is also a worry, particularly during hospital building. Sarcoptes scabies (scabies) is an ectoparasite that has caused outbreaks in health care on several occasions.

Diagnosis

The diagnosis and identification of hospital–acquired infection involves interpretation of clinical and laboratory findings. Clinically, a patient is assessed based on clinical sign and symptoms developed due to the infection. Pain, soreness, redness, localized swelling, and purulent discharge from the wound are symptoms of a superficial incision site infection. The patient developed a fever (> 38°C), localized discomfort or tenderness, and purulent discharge from the incision if the infection was at a deep cut. Fever (>38°C), urgency, frequency, and dysuria were all reported in a patient with symptomatic UTI. However, patients under the age of one year may have hypothermia (37°C), apnea, bradycardia, lethargy, or vomiting.

The pathogen was isolated by urine culture, which was used to make the laboratory diagnoses. The amount and

types of bacteria in the urine must be determined as part of the diagnostic process. If a mid–stream urine culture includes 105 organisms per ml and no more than two types of microbes, it is deemed positive.

Nosocomial infection prevention

It is the responsibility of all individuals and services providing health care to prevent nosocomial infections. In addition, everyone must work together to limit the risk of infection for both patients and employees. Although not all hospital infections are preventable, the majority of them can be. Surveillance of NIs is an important aspect of infection control, and it is widely recognized as a first step toward prevention around the world. Reduced health–care–associated infection rates, on the other hand, is dependent on a number of factors. Staff–related procedures, particularly hand hygiene, have recently received a lot of attention. Furthermore, there has been a growing understanding that environmental controls should be an important part of any overall plan for preventing health–care–associated illnesses [2].

Hand washing is still the most critical action in infection prevention. Gloves, gowns, and masks have a role in avoiding infections, but they are frequently misused, resulting in unnecessary service expenses. Many people are visibly disturbed when their inadequate hygiene practices are revealed, and many are outraged when it is claimed that they could be disease vectors, spreading dangerous bacteria among their patients, complicating infection control efforts [2].

Materials and Method

Study area and period

The current research was carried out at the Attat Specialized Hospital, which is located 175 kilometers southwest of Addis Ababa, 17 kilometers from the town of Wolkite on the road to Hosanna in the Cheha Woreda of the Gurage Zone, SNNPRs, Ethiopia. Since its establishment in 1969, the hospital was managed and run by Medical Mission Sisters under the Eparchy of Emdibir. According to medical mission sisters, the hospital has ten wards (0–9) providing extensive integrated health services for more than 800,000 people with in and out of the operational area. Between April and June 2018, samples were evaluated at Wolkite University's Department of Biotechnology and Biology laboratory, which is part of the College of Natural and Computational Science.

Study design

A cross–sectional descriptive study was conducted using a structured questionnaire and specimen assessment from the stethoscope of Attat Hospital healthcare workers.

Study population

All healthcare personnel (doctors, nurses, health officers and students) having their stethoscope on–hand during data collection constitute the source population for the study. According to the report from the medical director of Attat hospital, they had around 50 stethoscopes for 10 wards, which were used as the sampling frame. A proportional sample size was determined for each department (including inpatients and OPD) and participants were selected using a simple random sampling.

Sample size

There are 26 stethoscopes required for sample collection from different wards and from different professionals.

Selection criteria

Healthcare personnel who were willing to give informed consent and the study included anyone who had their personal stethoscope on hand at the time of data collection. The study did not include those who:

- Do not have a stethoscope on hand during data collection.
- Already participated in the study while working in another ward.
- Refuse to give informed consent.

Data and sample collection

The investigators went to any inpatient or outpatient department for data and sample collection without any prior notice. Then, after obtaining consent, self–administered questionnaires were utilized to gather socio––demographic data characteristics (gender, profession and experience) of participants, use of stethoscopes, stethoscope cleaning habits, and perceived barriers to cleaning. Swam samples for bacteriological and antibiotic resistance profiling were taken from the diaphragmatic section of stethoscopes using a sterile cotton–tipped applicator bathed in sterile normal saline (0.85 % w/v).

The obtained swabs were immediately placed in Amies transport media, and samples were sent to the laboratory in an ice box with proper and comprehensive labeling, along with the questionnaire.

Bacterial Pathogen Isolation, Enumeration, and Identification

The material was inoculated in duplicate on Blood agar, Tryptone soya agar, and MacConkey agar and incubated aerobically at 370C for 48 hours after gentle mixing. The media were inspected for bacterial growth after incubation, and the total number of colonies for each sample was tallied. Significant growth was defined as a colony count of more than 20cfu/diaphragm [29], and the stethoscope was deemed contaminated. In tryptone soy broth and agar slant, representative colonies from contaminated stethoscopes were purified and stored. Following normal bacteriological techniques, the isolates were identified to the genus and species level.

Based on colony characteristics (appearance, size, and color), cell morphology, Gram reactions and KOH test obtained further identification of bacteria was made by a series of biochemical tests. Mannitol salt agar and blood agar plates were used to cultivate Gram–positive cocci. Following that, catalase and tube coagulase tests were performed. Staphylococcus aureus was identified in isolates that passed all three tests. Isolates tested negative for tube coagulase was considered coagulase–negative staphylococci (CoNS).b Catalase negative gram positive cocci were cultured on blood agar and pattern of hemolysis (alpha, beta, and gamma) was observed.

Antibiotic sensitivity test

The following antibiotics were used to determine the anti biogram of the isolates: Penicillin (10μg), Chloramphenicol (30μg), Ciprofloxacin (5μg), Cefoxitin (30μg), Cotrimaxozole (25μg), and Vancomycine (30μg).The antibiotic discs were selected based on availability and current use in health facilities of Ethiopia. Direct colony suspension of the test organism in sterile saline solution were prepared, the turbidity of the inoculum was adjusted to a 0.5 McFarland standard (1.5x108CFU/ml). A new sterile cotton–tipped swab dipped in the suspension was used to wipe the surface of Mueller Hinton agar plates within 15 minutes of inoculum formation. Then, within 15 minutes of inoculating the MHA plate with sterile forceps, a set of 6 standard antibiotic discs was applied. MHA plates were incubated at 370C for 18–24 hours, and the diameter of each antimicrobial disc's zone of inhibition was determined [8].

Data analysis

The SPSS v23 computer software was used to enter and evaluate the data. Categorical variables were displayed in tables and bar graphs, with frequencies and percentages summarized. A statistically significant difference was defined as a P–value of less than 0.05.

Results

Study participants' socio–demographic profiles

A total of 26 stethoscopes were tested for bacterial contamination by four separate professionals from different hospital wards. Medical ward (IPG & OPD) (8), Gynecology ward (6), Surgical ward (IPD&OPD) (5), Pediatrics ward (IPD&OPD) (3), Emergency ward (2), Neonatal (1), and Delivery ward (1) were among the 10 wards where these health professionals worked (1).

The study included equal number of males (13) and females (13), where the majorities (10, 39%) were doctors, followed by nurses (7, 27%), medical students (5, 19%) and health officers (4, 15%).Most of the participants years of experience were less than 2 years (11, 42%), followed by 2–5 years (9, 34), and 5–10 years and >10 years (3, 12% each) as shown on (Table1).

Table 1 – Health–care personnel's socio–demographic features at Attat Hospital, Wolkite.

		UNCONTAMINATED	*CONTAMINATED*	*Total (%)*
		Count (%)		
GENDER	***Male***	6 (75)	7 (39)	***13 (50)***
	Female	2 (25)	11 (61)	***13 (50)***
PROFFESSION	***Doctor***	4 (50)	6 (33)	***10 (39)***
	Nurse	1 (12.5)	6 (33)	***7 (27)***
	Student	2 (25)	3 (17)	***5 (19)***
	Ho	1 (12.5)	3 (17)	***4 (15)***
EXPERIANCE	***<2Yrs***	4 (50)	7 (39)	***11 (42)***
	2–5Yrs	3 (38)	6 (33)	***9 (34)***
	5–10Yrs	0	3 (17)	***3 (12)***
	>10Yrs	1 (12)	2 (11)	***3 (12)***
WARD	***Medical***	2 (25)	6 (33)	***8 (31)***
	Gynecology	2 (25)	4 (22)	***6 (23)***
	Surgical	1 (12.5)	4 (22)	***5 (19)***
	Pediatrics	2 (25)	1 (6)	***3 (11)***
	Emergency	1 (12.5)	1 (6)	***2 (8)***
	Neonatal	0	1 (6)	***1 (4)***
	Delivery	0	1 (6)	***1 (4)***

Stethoscopes: knowledge, attitudes & practices (KAP) survey

Of 26 stethoscopes studied, almost one third (29%) of the owner reported that the last time they cleaned their stethoscope was last week. Other responded cannot recall (20%), never (17%), today (17%) and yesterday (17%). When asked how often they clean their stethoscope, the majority of respondents (23%) said once daily, followed by an equal number of respondents (19%) who said every patient, once weekly, or numerous times a day. Fewer (12% and 8%) responded rarely if ever and never, respectively. The agents they used for cleaning their stethoscope were alcohol wipes (92%) and antiseptic wipes (8%). No significant relation was identified among the use of disinfectant and bacterial contamination.

With regards to the ideal frequency of cleaning, 69% responded cleaning before and after every patient would be the best approach to keeping the stethoscope clean.81% of the participants believe that stethoscope could transmit infectious agents. For 19% of participants, cleaning their stethoscope at the start and end of the day was sufficient, while 12% had no notion what the appropriate frequency of cleaning. Forgetfulness (46%), lack of time (18%) (18%) were identified as the major barrier of cleaning stethoscopes. Concern for damage and lack of supplies were the other barriers to cleaning.

Bacterial contamination of stethoscope diaphragm

After two days of incubation, the diaphragms of all stethoscopes examined from ten wards revealed varying degrees of bacterial contamination. 18 (69.2%) of the 26 stethoscopes tested were heavily contaminated (>20 CFUs/diaphragm), while the other (30.8%) were not.

From 18 contaminated stethoscopes, 11 (61%) were from female health care personnel and 7 (39%) were from male. In terms of profession, the most frequently contaminated stethoscopes were those used by doctors (6, 33%) and nurses (6, 33%), followed by medical students and health officers. While analyzing the proportion, majority of stethoscopes used in Medical (6, 33%), Gynecology (4, 22%) Contamination was found in the Surgical ward (4, 22%). On stethoscope diaphragms from emergency, pediatrics, delivery, and neonatal wards, there was significantly less contamination (**Table 2**).

Table 2 – Level of bacterial contamination in terms of gender, profession, experience and ward.

		UNCONTAMINATED	CONTAMINATED	Total (%)
		Count (%)		
GENDER	Male	6 (75)	7 (39)	13 (50)
	Female	2 (25)	11 (61)	13 (50)
PROFFESSION	Doctor	4 (50)	6 (33)	10 (39)
	Nurse	1 (12.5)	6 (33)	7 (27)
	Student	2 (25)	3 (17)	5 (19)
	Ho	1 (12.5)	3 (17)	4 (15)
EXPERIANCE	<2Yrs	4 (50)	7 (39)	11 (42)
	2–5Yrs	3 (38)	6 (33)	9 (34)
	5–10Yrs	0	3 (17)	3 (12)
	>10Yrs	1 (12)	2 (11)	3 (12)
WARD	Medical	2 (25)	6 (33)	8 (31)
	Gynecology	2 (25)	4 (22)	6 (23)
	Surgical	1 (12.5)	4 (22)	5 (19)
	Pediatrics	2 (25)	1 (6)	3 (11)
	Emergency	1 (12.5)	1 (6)	2 (8)
	Neonatal	0	1 (6)	1 (4)
	Delivery	0	1 (6)	1 (4)

Phenotypic characteristics of bacterial isolates

Based on colony appearance, size, and color, 13 (72%) of the contaminated stethoscope showed a single, uniform colony growth, while the rest (5, 28%) had polymicrobal growth. Of which all colonies identified were gram–positive organisms, while no gram–negative bacteria were observed. A total of twenty–five representative bacterial colonies were primarily selected, in which twelve distinct isolates were purified and preserved for further investigation.

The selected isolates had cultural characteristics that were similar and different, and they were identified to genus level based on their phenotypic and biochemical characters, as well as hemolysis on blood agar and mannitol salt agar for fermentation analysis. The results are presented in **Table 3 and Fig 1**. Among the isolates identified, *Staphylococcus aureus* constitutes38.5%. Coagulase negative *Staphylococcus, Streptococcus* sp. and *Bacillus* sp. constitute 26.8%, 23.2% and 11.5% for the respective bacterial isolates (Fig 1).

For the isolation and identification of staphylococcus aureus from stethoscope samples that had previously grown in blood agar and tryptone soya agar, mannitol salt agar (MSA) was utilized as a selective and differential medium. This media was selective for staphylococcus aureus which ferment mannitol and produce yellow colonies with yellow zone around the colony; non mannitol fermented bacteria remain red to pink and colorless in the medium (Figure 3). The majority of the isolates (38.5%) were show yellow zone in the medium and identified as S. aureus.

The action of bacterial hemolytic exotoxins on red blood cells was used to identify normal flora from pathogenic bacteria using a blood agar plate (BAP) as a bacterial growth medium. The isolates were described and identified as streptococcus species based on their hemolytic (Alpha hemolysis, Beta hemolysis, and Gamma hemolysis) patterns (Figure–4). Streptococcus pyrogenes was identified as the bacterium that caused beta hemolysis on blood agar. On BAP, alpha hemolysis indicated the growth of normal flora, while gamma hemolysis suggested that the growth on BAP had no effect on the agar's appearance. Streptococcus pyrogenes (13.2%) was identified from the isolates and streptococcus pneumonia (10%) as normal flora as it showed gamma hemolysis in BAP.

Table 4 shows bacterial colony counts by gender, profession, experience and ward. The mean colony count of different wards was 109, where the highest (220) and lowest (36) was recorded in Delivery and Emergency ward, respectively. The data also showed difference between female (151) and male (67) mean colony count, which showed a significant difference at $P<0.05$. Nurses had the greatest mean colony count (148), while doctors had the lowest (79). In contrast, a non–significant mean difference was found in respect to health care personnel's years of experience.

Figure 1 – Bacterial profile isolated from stethoscope diaphragm.

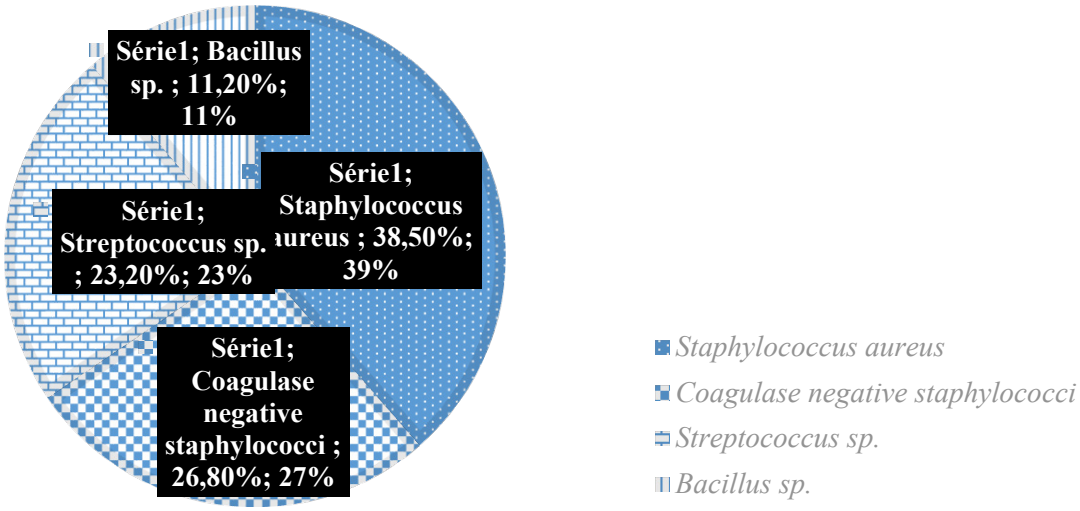
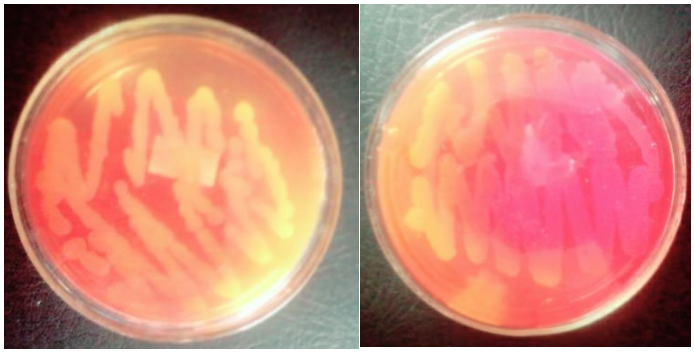
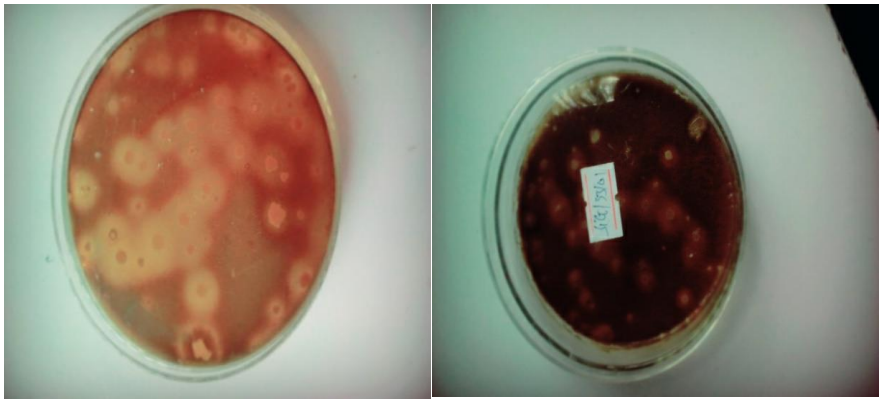


Figure 2 – Image of bacterial isolates that grown in mannitol salt agar.



Staphylococcus aureus ***Staphylococcus epidermis***

Figure 3 – Image of hemolytic streptococcus grown in blood agar.



Streptococcus pyrogenes ***Streptococcus pneumonia***

Table 4 – Bacterial colony counts from culture of stethoscope diaphragm surface.

		MEAN BACTERIAL COUNT
GENDER	**Male**	67.7
	Female	150.8
PROFFESSION	**Doctor**	79.2
	Nurse	148.6
	Student	112.6
	Ho	111.3
EXPERIANCE	**<2Yrs**	111.1
	2–5Yrs	111.7
	5–10Yrs	74.3
	>10Yrs	130
WARD	**Medical**	135.6
	Gynecology	106.7
	Surgical	140
	Pediatrics	36.7
	Emergency	22.5000
	Neonatal	40
	Delivery	220

Patterns of antimicrobial susceptibility in isolates

The isolates' antibiotic sensitivity pattern was examined for the following antibiotic discs: co–trimoxazole, chloramphenicol, penicillin, vancomycin, ciprofloxacin and cefoxitine. All isolates were susceptible to the co–trimoxazole and ciprofloxacine, while resistant to cifoxitine. They showed intermediate growth against vancomycine. All except streptococcus were found resistant against penicillin. Both S. aureus and CoNS were sensitive to the chloramphenicol; Streptococcus was intermediate while bacillus was resistant to the chloramphenicol (**Table 5 & Figure–4**).

Table 5 – Bacterial isolates from stethoscopes were tested for antimicrobial sensitivity.

ANTIBIOTIC DISCS	TYPE OF ISOLATES			
	S. aureus	***CoNS***	***Streptococcus sp.***	***Bacillus sp.***
Co–trimoxazole (25 µg)	S	S	S	S
Vancomycin (30 µg)	I	I	I	I
Cifoxitine (30 µg)	R	R	R	R
Penicillin (10 µg)	R	R	I	R
Ciprofloxacin (5 µg)	S	S	S	S
Chloramphenicol (30µg)	S	S	I	R

Key:R=Resistant; S=Susceptible; I=Intermediate

ANTIBIOTIC DISCS	TYPE OF ISOLATES WITH THEIR DIAMETER(CM)			
	S. aureus	**CoNS**	***Streptococcus* sp.**	***Bacillus* sp.**
Co–trimoxazole (25 µg)	2.4	3.1	2.5	3.2
Penicillin (10 µg)	1.3	1.5	1.7	1.3
Cifoxitine (30 µg)	1.2	1.5	1.3	1.4
Vancomycin (30 µg)	1.67	1.7	1.7	1.9
Ciprofloxacin (5 µg)	3.5	2.9	2.8	3.2
Chloramphenicol (30µg)	3.6	3.00	1.7	1.3

Figure 4 – Antimicrobial sensitivity test result (inhibition zone and diameter measurement).

Discussion

The stethoscope is a piece of medical equipment that is utilized by all health–care workers. Our research indicated that 69.2 percent of the stethoscopes surveyed were infected, which is similar to previous findings that found 71 percent to 100 percent of stethoscopes were colonized by different bacteria [7, 29].

Doctors' and nurses' stethoscopes were found to be more polluted (33 percent apiece) than those used by other health workers. This research is comparable to that of [7] The fact that doctors and nurses use stethoscopes more frequently than other health care staff may explain the higher rate of bacterial colonization, even if the difference was not statistically significant [19, 7]. Nurses, on the other hand, had a greater mean microbial load (149) and medical students (113) and the lowest were recorded in doctors (79), which might be due to improved stethoscope cleaning habits in later case.

In this investigation, a swab of stethoscopes taken from clinicians in the medical ward (8) revealed the highest level of infection. Medical physicians may wear stethoscopes more frequently than others, which could explain why they have a greater prevalence of bacterial contamination.

A total of 25 colonies were isolated from 18 (69.2%) contaminated stethoscope diaphragms, although only 13 unique bacterial isolates were examined for further phenotypic characterization. Surprisingly, no gram–negative bacteria were found in any of the stethoscope diaphragms that were analyzed. Gram–positive bacteria were found in all of the isolates.

For bacterial growth and enumeration, blood agar, MacConkey agar, and tryptone soya agar media were utilized. Gram positive bacteria from four different species were recovered from both blood and tryptone soya agar. The largest number of bacterial isolates per diaphragm was three, while the lowest number was one. The majority of the isolates (40%) were identified to be potential pathogens. Staphylococcus aeurus species was the most prevalent isolate (38.5%), followed by coagulase negative staphylococci, although Staphylococcus epidermis and enterobacteracea were the most common organisms recovered from stethoscopes in investigations by[7]. Co–trimoxazole and ciprofloxacine were determined to be effective against all gram–positive isolates based on their resistance profiles. Meanwhile cifoxitine and penicillin were not. That is all isolates were susceptible to the co–trimoxazole and ciprofloxacine, while resistant to cifoxitine. They showed intermediate growth against vancomycine. All except *streptococcus* were found resistant against penicillin. Both *S. aureus* and *CoNS* were sensitive to the chloramphenicol; *Streptococcus* was intermediate while bacillus was resistant to the chloramphenicol.

In this study, a questionnaire was used to analyze participants' knowledge, attitudes, and practices regarding the role of stethoscopes as carriers of infectious organisms. We found that stethoscopes were contaminated with dangerous germs and that inadequate stethoscope cleaning/disinfection techniques were related with high contamination. In particular,34 stethoscopes cleaned on the same day as the data collection were uncontaminated, compared to 100 percent contamination in those who said they never/cannot recall. Because even brief contact with a patient's skin and the stethoscope can result in bacterial translocation[1], measures to reduce bacterial contamination through better stethoscope cleaning habits are needed.

Disposable stethoscopes, especially in clinical high–risk contexts, and the placement of a single–use silicone membrane over the stethoscope head to provide a prophylactic barrier have been proposed as ways to reduce infection transmission from stethoscopes[23]. Although these measures could reduce the risk of infection transmission via stethoscope, they are out of reach for the majority of health workers and health facilities in developing nations, including Attat Hospital. Instead, hospitals should implement more stringent stethoscope disinfection programs and practices as a standard of care [28]. Health personnel who strictly follow stethoscope disinfection procedures will reduce cross–contamination and increase patient safety in hospitals.

Conclusion

The current study found a higher percentage of bacterial contamination on the stethoscope diaphragm, indicating that there is a risk of nosocomial pathogen transmission. Many of the bacteria found in our study's stethoscopes (e.g., Staphylococcus aureus, CoNs; S.epidermis, *Streptococcus sp.; S.pyrogene*, and *Bacillus subtilis*) were known to cause serious infections in hospitalized patients. Staphylococcus and Bacillus species showed increased resistance to the drugs tested, however Streptococcus species did not. Infected stethoscopes were discovered in all parts of the hospital and among all types of medical professionals. The study also suggests that hospital employees should be alerted and educated about the potential health concerns linked with medical equipment.

To reduce infection transmission through stethoscopes, various techniques have been proposed, including the use of disposable stethoscopes, particularly in clinical high–risk areas, and the placement of a single–use silicone membrane over the stethoscope head to establish a prophylactic barrier. Although these measures could reduce the risk of infection transmission via stethoscope, they are out of reach for the majority of health workers and health facilities in developing nations, including Attat Hospital. Instead, hospitals should implement more stringent stethoscope disinfection programs and practices as a standard of care. Health personnel who

strictly follow stethoscope disinfection procedures will reduce cross–contamination and increase patient safety in hospitals. Training and motivating health care providers to put their knowledge into practice could be the next step in drastically lowering the bacterial load from the stethoscope, which would immediately reduce cross–contamination and improve patient safety in the hospital setting.

The Study's Limitations

The sample size was tiny (26 people), and it came from only one hospital. The frequency with which the stethoscopes were used differed from one participant to the next. In this investigation, the colonization of stethoscopes was not linked to hospital–acquired illnesses. Other contaminants such as anaerobic bacteria, fungi, and viruses were not investigated. The length of time the stethoscope was in touch with the patient's skin/clothing was unknown. Bacterial identification was done using phenotypic characterization, which is not as reliable as molecular approaches. The identification of such contaminating organisms and their role as nosocomial infections should be the focus of future research.

Recommendation

- The bacterial four isolates types of microbes need to be fully characterized using molecular techniques.
- Further study is important to identify other microbes from large enough sample size of different wards with their drug sensitivity tests is needed.
- Design instrument processing of stethoscope like other health service instruments
- There is a need of training for health personnel to increase the culture of decontaminations of their stethoscope.

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Comparison between three decellularization protocol whit SDS in swine esophagus: a future option in veterinary medicine

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Abstract: Disease such as megaesophagus and esophagitis does not have a cure in animals. Esophagitis has treatments depending on it's degree, while megaesophagus has a palliative treatment, which can be recommended euthanasia in cases of a poor prognosis, cases that the esophagus transplant could be at use, although it is not described in literature. In this perspective, tissue bioengeneering has techniques to create a minor immune response in transplants, as it removes the organ's genetic material, preserving the extra cellular matrix (ECM). Different protocols have been evaluated for swine esophagus decellularization, using sodium dodecyl sulphate (SDS) in concentrations 0,5%, 1,0% and 1,5% for seven days, analysing the remaining EMC integrity. It has been concluded that the material in 0,5% of SDS showed more integrity of the remaining EMC compared to the other concentrations, demonstrating that it is the best biological scaffold for future medical applications

Keywords: Decellularization. Tissues Bioengineering. Megaesophagus. Regenerative Medicine.

Introduction

According to König[16] (2016), the esophageal structure contains four layers: adventitia, muscular, submucosa and mucosa; Diseases like megaesophagus and esophagitis are the main diseases that affect animal esophagus. Esophagitis is more common in dogs than in cats1, consisting in the damage to the esophagus mucosa, in some cases affecting all the way to the submucosa and the muscular layer, depending on the severity and the agent that caused it[2]. Megaesophagus consists of dilation and esophageal hypomotility, which can be idiopathic, congenital or secondary to other disease[3].

Megaoesophagus's etiologies are diverse, being congenital idiopathic, acquired idiopathic or secondary to other diseases. The main symptom is regurgitation, that can occur minutes or hours after the ingestion of food, its frequency varies, occurring weekly or daily3. Complications of the megaesophagus are inhalation pneumonia, and may lead to cough, fever, weight loss or even death4. The treatment is palliative, aiming for a greater absorption of nutrients in the gastrointestinal tract and a better quality of life. In general, it consists of a specific diet for each patient, with medication for the primary disease when necessary[3]. These dogs' feeding is made in a 45° angle or greater if possible, counting with gravity's support for the food to pass through the esophagus to the stomach[4].

Esophagitis main prompter is gastric reflux during procedures with anesthesia. Chronic emesis can cause the disease in case of a high intensity and frequency2. The treatment in medium cases consists of nutritional management and light antacids. In more advanced cases, the treatment counts with nutritional management, more powerful antacids, or even the removal of the ill fragment through a partial esophagectomy[5]. These procedures can occur only in small portions of the organ, being indicated that the surgical removal be in the gap of 3 and 5 cm, resulting in surgical dehiscence in major portions[6].

Since a complete esophagectomy is not viable nowadays, techniques for situations of a major affected portion of the organ were created. Examples are the use of small intestine's submucosa for repairing lessons of the cervical esophagus[7], and the substitutions of the esophagus for a gastrotube using gastroplasty[8]. Although the techniques are effective, they can not completely replace esophagus function, which can lead to a bad nutrition after the operation and post-surgical complications[9]. Esophageal transplant could be an option in these cases, even though it is little described in literature in veterinary medicine.

As an option for transplants the regenerative medicine with advanced tissue engineering techniques. Tissue and organ decellularization techniques were and still are developing, aiming a minor immune response in procedures such as organs transplants. A tissue as a whole contains cells and extracellular matrix (ECM). The ECM has

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biomechanical properties, besides being responsible for cell survival . Decellularization is the removal of cells from a tissue or organ, resulting in the preserved ECM, which is named "biological scaffold". This process preserves the ultrastructure, biological activities and mechanical integrity from the tissue. The biological scaffold is very relevant for regenerative medicine, because it can be used in allogeneic transplants or even xenogeneic transplants, causing a minor immune response in the receptor [10–11].

Several decellularization protocols were already described, each one based on the type of tissues that compose the organ, amount of cells, density, lipidic content and other factors. Decellularization methods can be chemical, as an example detergents, biological, such as enzymes or physic, like temperature[10,11].

This study has the goal of suggesting and evaluating a swine esophagus decellularization protocol, aiming for its possible application in esophageal transplants surgeries in veterinary medicine.

Methodology

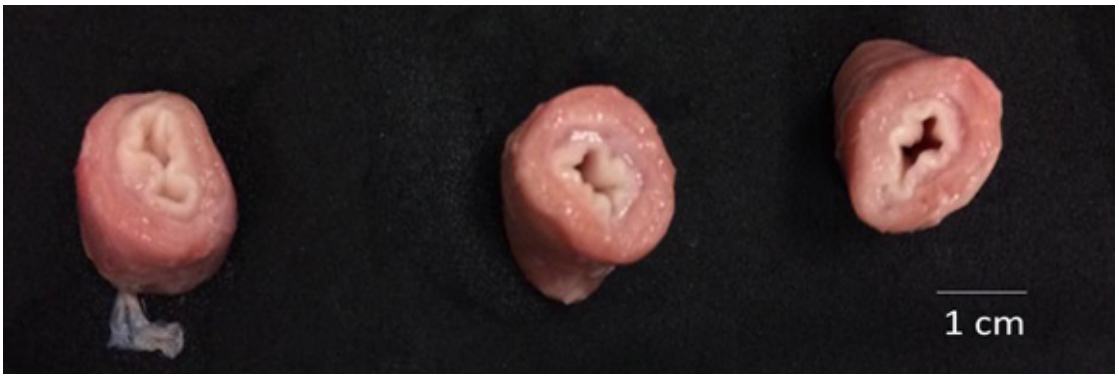
Ten (1 control and 9 samples for experimental design) swine esophagus fragments, of both sexes, weighing approximately 50g were obtained from the slaughterhouse in the city of São José do Rio Pardo–SP, in accordance with the Animal Experimentation Ethics Committee 1166080618. These Esophageal fragments were frozen at –80oC for at least 24 hours for storage and aid of cell lysis.

The organ was submerged in a phosphate buffered saline solution (PBS,136,9 mM de NaCl, 26,8 mM de KCl, 14,7 mM de KH2PO4 e 8,1 mM de Na2HPO4.7H2O, pH 7,2) followed by distilled water for cleaning it. Decellularization was made with sodium dodecyl sulphate (SDS), which is vastly used for this procedure[10–14]. Three different protocols were established using the concentrations of 0,5%, 1% and 1,5% SDS, combining a physical method (agitation) and a chemical method (use of ionic detergent) with different concentrations. The decellularized oesophagus were washed before each protocol began with distilled water for 5 minutes. During the decellularization protocol, they were submerged in PBS 1x once a day for 5 minutes, for sinking in distilled water afterwards, returning then to SDS immersion in its concentrations in the shaker. All of them suffered through this process for seven days straight. At the end, the samples were fixed in 4% paraformaldehyde buffering during 48h, for microscopic analysis. Afterwards, they were dehydrated in ethanol, diaphanized in xylol and soaked in paraffin. Microsections of 5 μm were made using a microtome(# RM2265, Leica – Nussloch, GE) and transferred for glass blades. The blades were immersed in haematoxylin and eosin (HE) and Masson Trichome (TM) for the presence of nucleus and EMC collagen evaluation. Beside these analyses, the scanning electron microscopy technique was used5.

Results

The samples used in the decellularization protocol were previously photographed for analysing the macroscopic aspect, as it is shown in the figure 1.

Figure 1 – Samples before decellularization protocol.



Source: Own autorship, 2018.

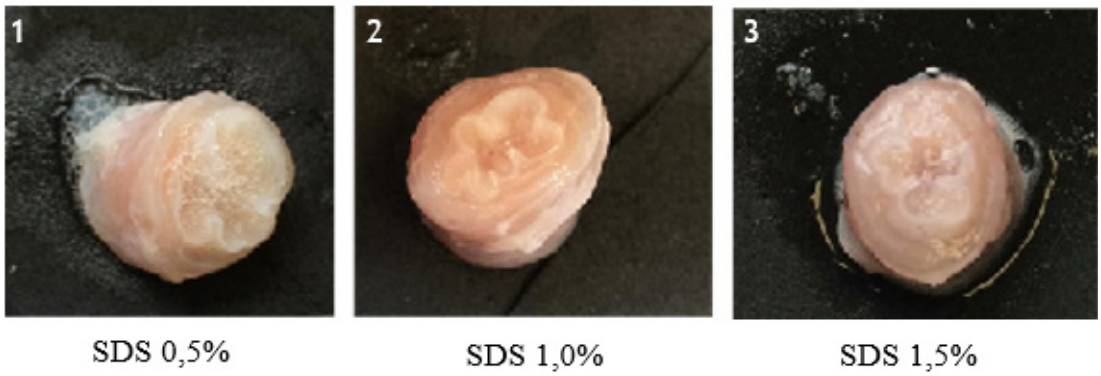
The EMC varies in quantity and composition according to each tissue, being more present in tissues such as connective tissue, and minor amounts in epithelial, muscular and nervous. ECM represents the matter secreted by the resident cells, being responsible for the cells survival and may influence in their proliferation and differentiation. In a general form, ECM is composed of fibrous proteins, such as collagen and elastin. It is also composed of glycosaminoglycans, proteoglycans and glycoproteins[10,18].

Subsequently to the decellularization protocol, the samples were photographed for efficiency evaluation. All the samples were "transparent", which is a macroscopic indicative that the decellularization process occurred10. The translucent aspect is due to the removal of cells from the tissue, taking away the primary colour. The translucid aspect can be observed in Figure 2.

The macroscopic analysis was unclear, since all the samples showed a preserved structure and a decellularized aspect. After noticing that, a light microscopy was made. In order to analyse the fragments searching for nucleus, the coloration Haematoxylin–Eosin (HE) and Masson Trichome (MT) for collagen presence methods were used.

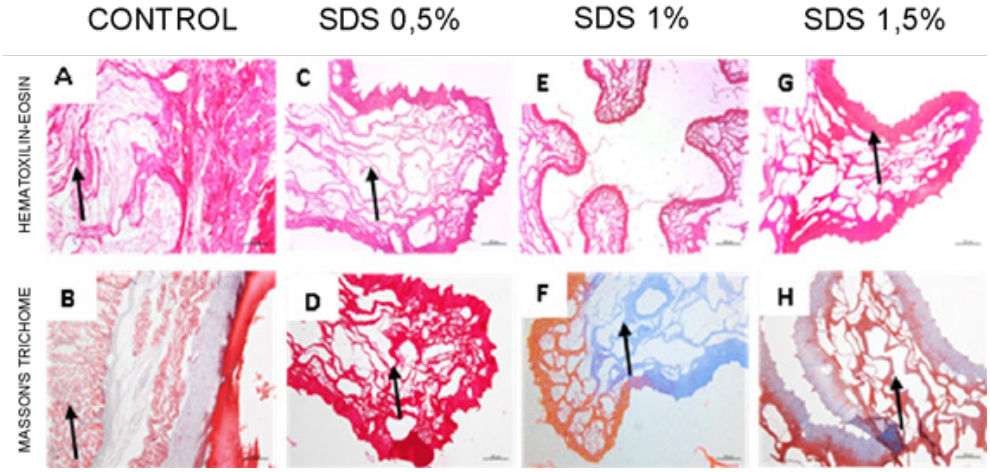
Haematoxylin is a dye in basic character, that paints acids structures in purple. The main example is the nucleus, containing DNA and RNA, both being acids. Eosin is an acid dye, painting basic structures in pink. It paints, among other things, EMC proteins18. TM is composed of haematoxylin, acid fuchsin, xylidine ponceau and green–blue, being used for giving colour to muscular tissue and collagen. The final results are: nucleus painted in brown; keratin, cytoplasm and muscular fibres in red; background as light green; collagen in blue or green19. The following image shows the light microscopies obtained from the control and the decellularized samples (Figure 3).

Figure 2 – Samples after decellularization protocol of 7 days. 1– Sample decellularized with SDS0,5%. 2– Sample decellularized with SDS 1%. 3– Samples decellularized with SDS 1,5%.



Source: Own authorship, 2018.

Figure 3 – A– Control coloration HE, whit mucosa intact (arrow). **B**– Control coloration MT, whit muscular layer (arrow). **C**– SDS 0,5% coloration HE, mucosa's EMC is preserved. **D**– SDS 0,5% coloration MT, muscular EMC is preserved. **E**– SDS 1% coloration HE, does not show nucleus, but EMC is poorly preserved. **F**– SDS 1% coloration TM. **G**– SDS 1,5% coloration HE, does not show nucleus, but EMC is poorly preserved (arrow). **H**– SDS 1,5% coloration MT, mucosa and muscular poorly preserved.



Source: Own authorship, 2018.

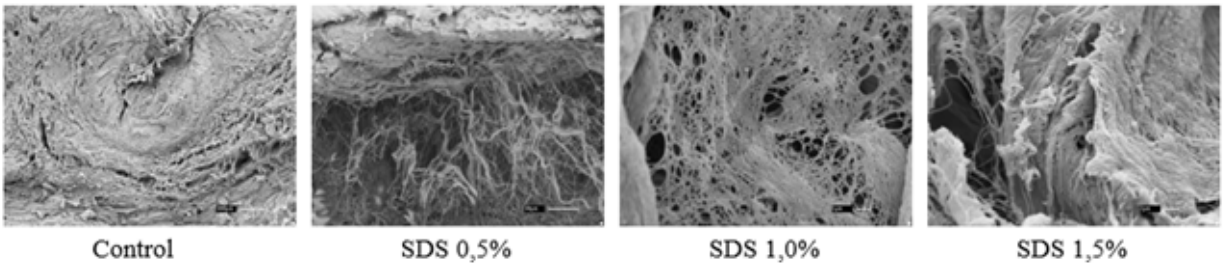
All the samples were decellularized, not showing nucleus in any of them. As the ECM, evidenced by TM, there is a better preservation in the SDS 0,5% sample compared to the others, showing a better preservation of the ECM, painted in red. The samples decellularized using SDS 1,0% and 1,5% although presented no nucleus presence, showed a poor ECM preservation.

The SDS 0,5% sample has presented a better ECM preservation, demonstrated in pink in HE colouring and red in TM. The preservation of this sample was considerable in the mucosa and muscular regions when compared to others. Neither HE nor TM presented nucleus in the samples, which is a visual indicative of the decellularization process. Added to the macroscopic analysis, where the samples presented a transparent aspect, it has been concluded that the sample subjected to the protocol of ionic detergent SDS 0,5% was the best between the samples evaluated.

It is important to understand the basic ECM constitution, which is a set of fibrous proteins, like collagen and elastin, and also extended glycoproteins, such as fibronectin and laminin, responsible for the adhesion cell–ECM[20]. That information is important for qualifying the microscopic analysis. Since collagen is the most abundant protein and it has a structural function, its evaluation is very important for the decellularization process.

The microscopic analysis is a bidimensional model, it provides a poor representation of the ECM components organisation. Therefore, a scanning electron microscopy technique was required. This analysis provided the subsequential image.

Fugure 4 – Scanning electron microscopy. Control – presents structural organisation of the esophageal tissue. SDS 0.5% shows the absence of cells with the best organised structure. 1% SDS shows a greater absence of cells and no structural organisation. 1.5% SDS also shows fewer cells and greater tissue disorganisation.



Source: Own authorship, 2018.

The control sample show's a preserved tissue, with cellular matter and a preserved ECM. Samples that were decellularized did not present cells, indicating that the decellularization process had succeeded. About the remaining ECM preservation, all of them had some level of degradation, being the 1,5% SDS sample the inferior preservation. Both 0,5% and 1,0% SDS samples obtained a satisfactory ECM ultrastructure preservation, although 0,5% SDS showed a better collagen fibres preservation.

Discussion

The decellularization protocol to be used depends on a lot of factors, such as quantity of cells in the tissue, density, lipidic content and thickness of the organ or tissue. The decellularization method can be physical, chemical or enzymatic[10,11].

Ackbar21 (2012) showed a decellularization protocol with SDS in sheep oesophagus, maintaining a good ECM preservation. Sitthisang[22] (2021) had good results in the decellularization protocol of swine oesophagus, using SDS detergent and a perfusion technique. These studies had satisfactory results, but the focus was human medicine, unlike this study, that aims a veterinary application.

Regarding clinical applications of this protocol, it is a future option for clinical trials. Although organ transplants are not much explored in veterinary medicine, it can be a valuable option for patients with poor prognostics, such as megaesophagus patients. In these cases, the treatment depends on the primary condition that is causing it, in most cases being idiopathic or neurological.

A 2020 study shows that in dogs, only 30,9% of the megaesophagus cases had a good outcome, while 19,8% had a persistence post treatment and 69,2% of the dogs died, either by secondary problems of the disease (49,9%) ou euthanasia (20,2%). This, along with other results in literature, shows the demand for a more efficient treatment for this disease.

Conclusion

Organs transplantation may be an option for future use, considering its advanced tissue engineering techniques as a resource for a minimum immune response in transplantations. For future use in veterinary medicine, the decellularization protocol using SDS 0,5% explained in this study is an option, although research are needed, such as the recellularization protocol, immune responses in vitro and in vivo tests, having swine esophagus as a proof of concept for the future use of canine esophagus decellularization in veterinary medicine for clinical use in cases of comprehensive esophagitis or megaesophagus,

the decellularization protocol using 0.5% SDS is a good option , although more studies are needed for future application.

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Bacterial cellulose/calcium alginate hydrogels in wound's cicatricial process of diabetic foot implementation: case report

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Abstract: It is well known that Diabetes mellitus (DM) is classified as a metabolic disease and in consequence several dysfunctions are expected, such as difficulties in wound healing process. Objectives: Due to the excellent properties of the developed bacterial cellulose/alginate hydrogel (BC/ALG), this material was applied to treat a diabetic ulcer patient. Method: The treatment was carried out at the Ambulatory of Santa Casa de Misericordia de São Carlos, by applying the hydrogel twice a week during 30 days. Results: After the period of treatment, the wound showed 84% of closure reduction when compared to its initial size Conclusion: Bacterial cellulose/ alginate hydrogel presented a high potential for successful treatment of wound closure.

Keywords: Wound Healing. Wounds and Injuries. Hydrogels

Introduction

Diabetes mellitus (DM) is characterized by a metabolic disease and due to the absence or inability of insulin to perform its function, several complications may occur including a rise in glycemic levels and with the evolution of the disease, other organ dysfunctions and permanent damage [1]. According to Brazilian Diabetes Society, there are approximately 13 million people living with DM, representing 6.9% of Brazilian population[2]. It is believed that in 2040 DM can affect up to 640 million adults aged 20–79 years worldwide[3].

Large concentrations of glucose in bloodstream can result in several metabolic complications, such as diabetic acidosis and non-ketotic hyperosmolar hyperglycemic syndrome, peripheral microangiopathy vascular disease, chronic cardiovascular disease (coronary, neurological, peripheral, renal, and retinal arterial disease) [4].

It is important to emphasize that one of the most common chronic complications caused by DM is the diabetic foot syndrome, which can be characterized by the presence of ulcers and infections, peripheral neuropathy, reduced physical mobility and in some extreme cases, the surgical limb amputation is required, which corresponds to 40 to 60% of non-traumatic amputations[5]. Despite being extremely refutable, the rate of amputations has been considered as a quality care indicator of diabetic foot complications [6].

However, Moretti et al. 2009[7] pointed out that in addition to foot ulcers leading to amputations and being considered the main causes of patient's disabilities, these factors can not be recorded as positive in relation to treatments.

According to Lima and Araujo, 2013[8] several mechanisms are identified as delay factors in healing process of diabetic patients, such as the excessive production of Reactive Oxygen Species (ROS), decreased Nitric Oxide (NO) and decreased Growth Factors (GFs) and proteins response in insulin signaling pathway. On the other hand, an ischemic microenvironment, associated with limited NO bioavailability and/or NO inactivation by ROS, leads to endothelial dysfunction characterized by the difficulty of arteries and arterioles regarding their functions in the regulation of vascular tone and adequate blood circulation. However, the presence of ROS in diabetic patients is usually the main cause that influences against to wound healing process.

In this context, Vieira et al 2017[9], emphasize the importance of new wound coverings and technologies in wound healing success. A clear and current overview of current evidence is required to facilitate decision-making regarding dressing use. Oliveira et al, 2020[10] highlighted that with the advancement of science and biotechnology the options of dressings available on the market exceeds 3000 types. In terms of composition, the most common devices are based in polymeric foam with or without silver, hydrocolloids, silver activated carbon, silver mesh, therapies based on growth factors, external tissue expanders, negative

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pressure and natural and synthetic biopolymers[10–11].

Despite of the available dressings diversity, new ones composed by natural biopolymers such as bacterial cellulose (BC) are welcome. Thus, BC obtained from Gram–negative bacteria fermentation in association with sodium alginate extracted from seaweed presented excellent properties such as less toxicity, biocompatibility and great wound healing progress when applied [12–13]. This fact could may be attributed to BC nanometer–sized fibers that mimic collagen which improves healing process resulting in reduced pain [14]. In addition, calcium alginate influences wound hemostasis by calcium ions release as the gel perform as a matrix for aggregating platelets and erythrocytes[15].

Recently Sulaeva et. al., 2020 applied BC/ Alginate membranes in wound treatment. the results showed that membranes expressed greater water retention capacity (up to 70 to 90%), kept the wound moist providing a better healing process. However, membrane dresses own some complication and disadvantages in handling during the exchange, which can cause discomfort to the patient. According to above mentioned properties, hydrogels based dressings represents the first choice and an ideal treatment of acute, chronic and diabetic wounds, minimizing infections with the bonus of easier removal [11].

Therefore, due to the excellent characteristics presented, this paper will report the use of BC/ALG hydrogel in a challenge wound closure treatment of a diabetic patient.

Methods

• Case Report

The Patient is a man, 63 years old, diabetic and hypertensive. His medical report revealed continuous use of specific medication supervised by frequent medical follow–up. It was also reported toe amputation in September 2019 and the presence of a diabetic ulcer in February 2020.

• Study location

The treatment was carried out at the Photodynamic Therapy unit of Santa Casa de Misericordia, São Carlos (Brazil).

• Treatment

The performed treatment protocol included asepsis of the lesion with 0.9% saline, followed by mechanical debridement by using a scalpel blade, when necessary. Thus, the wound was covered with the BC/ALG hydrogel (patent n ° BR 10 2019 021848 7), occluded with sterile gauze and bandage. This procedure was repeated twice a week, for 30 days. However, the mechanical debridement step was performed just when necessary.

Results

As mentioned before, healing process is highly complex, especially when related to a diabetic health condition, which requires special care both regarded to glycemic control, nutrition and rest, as well as direct care related to the extension of the injury. At this point, is important to consider all options available to choose the most appropriate dress that will provide better clinical performance in relation to tissue repair.

All measurements were obtained by using ImageJ software at 0, 7, 21 and 30 days, according to the pre–established time of study and converted to cm2 as shown in table 1. Each data result was carried out in triplicate and the media was calculated to each period of study. It was possible to observe a remarkable reduction of 84% of the wound area in just 30 days of treatment, when compared to the initial diameter size.

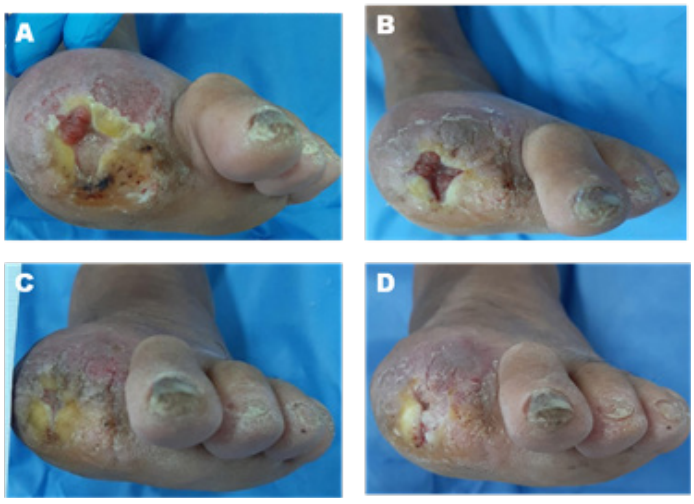
Table 1 – Data results of wound area size, measured at 0, 7, 21 and 30 days of treatment, expressed in cm^2.

Period of study	Wound size
0	2.344cm^2
7° day	2.332cm^2
21° day	0.830cm^2
30° day	0.377cm^2

Discussions

Figure 1 A shows the wound before treatment which is possible to observe hyperkeratosis at the edges of the lesion, some granulation tissue presented in wound bed, with 2,344 cm^2 of surface area. In 7th day of treatment, the wound appeared cleaner, without devitalized tissue presenting a slightly area reduction to 2,332 cm^2 (Figure 1B). Figure 1C presented the results of the 21th day of treatment, which was possible to observe a considerable wound closure that indicates that the hydrogel may be contributing to accelerate the healing process. Finally, at the and 30th day of treatment, the measurements of the wound area showed a significant reduction to 0.377cm2 (Figure 1 D).

Figure 1 – Clinical evolution of the healing process using the BC/ALG hydrogel as treatment. A) Initial; B) 7 days of treatment; C) 21 days of treatment and D) 30 days of treatment.



Some characteristics are usually involved in complex wounds, such as ischemia, necrotic tissue, excessive collagen accumulation, reduction of angiogenic factors, delay in inflammatory response which negatively influences the healing process. According to the images, clinical success in closure is an important data that indicates tissue repair. Therefore, the treatment performed with bacterial cellulose containing calcium alginate hydrogel may be a promising alternative for the treatment of complex wounds, since there was an acceleration in healing process and tissue repair of the treated diabetic wound.

Contribution to practice

It is possible to notice that the chosen treatment by using a BC/ALG hydrogel clearly promoted the closure in a challenge wound of a diabetic patient, improving the comfort and reducing the pain and injury exposure. All these factors acted into less wound contamination which probably influenced a faster wound closure. In addition, it is worth mentioning that there is no data related to this specific hydrogel used because it was recently patented by Seven Produtos Biotecnologicos – Ltda company which aims to commercialize it as soon as possible.

Conclusions

The results showed that it was possible to observe a considerable reduction of 84% in average when compared to initial wound size. This achievement may be attributed to the excellent biological properties of the applied hydrogel which highly improved the wound healing process. Despite more complementary histological data are needed to observe cellular tissue repair, it is possible to conclude that BC/ALG hydrogel configured a great option in terms of treatment of challenge diabetic wounds.

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