Research Article

Comparison of Bis-Plus-D and API 20 Strep for the Identification of Streptococci in the Laboratory of the University Hospital of Befelatanana Antananarivo Madagascar

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Abstract

Background: In medical analysis laboratories, techniques for identifying bacteria are currently becoming more and more numerous. The objective of this study is to compare the Bis-Plus-D and the API 20 Strep for the identification of streptococci

Methods: During the study period, 22 Gram-positive coci isolates were identified using Api 20 Strep and Bis-Plus-D.

Results: During the study period, 22 isolates of streptococci were identified simultaneously using Bis-Plus-D and Api 20 Strep. The streptococci identified were represented by Streptococcus anginosus, Streptococcus agalactiae, Streptococcus uberis, Enterococcus columbae, Streptococcus pyogenes, Streptococcus constellatus, Enterococcus durans Enterococcus casseliflavus, Enterococcus faecalis and Enterococcus faecium. This study showed 50% of discordant results between Bis-Plus-D and Api 20 Strep. Regarding the bacterial identification qualities, they were all excellent (100%) for Api 20 Strep. For Bis-Plus-D, the identification qualities were excellent for 36.4% of bacteria, very good for 36.4% of bacteria, good for 22.7% of bacteria and intermediate for 4.5% of bacteria. Concerning the probability scores for exact identification of bacteria, they vary between 82% to 100% for Api 20 Strep and between 62.6% to 99.9% for Bis-Plus-D.

Conclusion: Api 20 Strep remains the best identification bacterial system and can be used by all bacteriology laboratories for good identification of bacterial species.

Keywords: Bacteria; bacterial identification system; qualitative evaluation.

Introduction

The heterogeneous genus of streptococci plays an important role in human disease. Streptococci are estimated to cause 700 million human infections each year worldwide, with an estimated total of 500,000 deaths. Louis Pasteur recognized streptococci as one of the first microorganisms to cause contagious disease in 1879 [1]. Most streptococci are commensals, pathogens, or opportunistic pathogens for humans and animals [2]. It is therefore of immense importance to characterize and identify these bacteria. The prognosis of bacterial infections depends not only on the antibiotics used but also by the sensitivity of germs to these antibiotics [3]. The role of microbiology laboratories is to correctly identify streptococci and correctly perform the

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antibiogram in order to ensure proper management of the patient's infectious disease.

In developing countries like Madagascar, manual bacterial identification systems are still used. The majority of bacteriology laboratories in Madagascar use the Api 20 Strep bacterial identification system to identify the streptococci. However, the use of this kit has some drawbacks. Thus, it was decided to use other bacterial identification systems offered by the suppliers. This is the Bis-Plus-D. Before using the Bis-Plus-D, it must be compared with the Api 20 Strep. Thus, the objective of this study is to compare the 2 bacterial identification systems Bis-Plus-D and Api 20 Strep for the identification of streptococci.

Materials and methods

Type and period of study

This is an evaluative study by comparing the two bacterial identification systems Bis-Plus-D and Api 20 Strep over a period of 3 months from January 2022 to March 2022 in the laboratory of the University Hospital of Joseph Raseta Befelatanana.

Procedures

During the study period, 22 bacterial isolates were identified simultaneously with Bis-Plus-D and Api 20 Strep. Bis-Plus-D is a new bacterial identification system provided by Cypress Diagnostics and should be evaluated before use by comparing it with Api 20 Strep. Indeed, since the opening of the bacteriology laboratory of the University Hospital of Joseph Raseta Befelatanana. The Api brand bacterial identification systems supplied by Biomérieux have been the only ones to be used in the laboratory. Thus, Api 20 Strep represents the gold standard test in this study.

API reagent include 25 microtubes containing dehydrated substrates. Conventional tests are inoculated with a saline bacterial suspension which replenishes the media. The reactions produced during the incubation period are reflected by spontaneous color changes or revealed by the addition of reagents. The assimilation tests are inoculated with a minimum medium and the bacteria grow only if they are able to use the corresponding substrate. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to theReading Table and the identification is obtained by referring to the Analytical Profile Index or using the identification software. API 20 Strep is a standardized system combining 20 biochemical tests which have a high discriminating power. It makes it possible to make a group or species diagnosis for most streptococci, enterococci and for the most common related germs.

Concerning the Bis-Plus-D, its panel is made of 24 microtiter wells, each of them with different dehydrated reagents for a specific biochemistry test. Upon addition of bacterial suspension, the reagents in the well are constituted. During incubation, the metabolic activity of the inoculated bacteria is coupled to a certain colorimetric reaction depending on the specific test. A supplementary color code guide allows the user to determine whether every test is positive or negative. This, in last instance, depends on whether the bacteria from the inoculum possess or not a certain metabolic activity. Based on the acquired results profile, the identification of the bacterial species can be obtained by introducing the results profile in the Cypress identification software.

Study parameters

Study parameters were represented by bacteriological results on Bis-Plus-D, bacteriological results on API 20 Strep, the probability scores of exact identification of the bacterial species, the identifying qualities of streptococci, the concordance and discordance of the results, advantages and disadvantages of using Bis-Plus-D and API 20 Strep.

Ethical considerations

This study was authorized by the director of establishment of the University Hospital of Befelatanana and the Department Head of the laboratory before its implementation. This study respected the notion of anonymity and confidentiality. Otherwise, this study was carried out with the technical and financial support of the company Médical International of Madagascar and the Mérieux Foundation.

Results

Bacteria identified by Bis-Plus-D and Api 20 Strep during the study period

During the study period, 22 isolates of streptococci were identified simultaneously using Bis-Plus-D and Api 20 Strep. The streptococci identified were represented by Streptococcus anginosus, Streptococcus agalactiae, Streptococcus uberis, Enterococcus columbae, Streptococcus pyogenes, Streptococcus constellatus, Enterococcus durans Enterococcus casseliflavus, Enterococcus faecalis and Enterococcus faecium (table 1).

This study showed 50% of discordant results between Bis-Plus-D and Api 20 Strep (figure 1)

Bacterial identification qualities

Regarding the bacterial identification qualities, they were all excellent (100%) for Api 20 Strep. For Bis-Plus-D, the identification qualities were excellent for 36.4% of bacteria, very good for 36.4% of bacteria, good for 22.7% of bacteria and intermediate for 4.5% of bacteria (figure 2).

Probability scores for exact identification of bacteria

Concerning the probability scores for exact identification of bacteria, they vary between 82% to 100% for Api 20 Strep and between 62.6% to 99.9% for Bis-Plus-D (figure 3).

Table 1: Bacteria identified by Bis-Plus-D and Api 20 Strep

| Bacteria identified	Bis-Plus-D	API 20 Strep	Results
bacteria 1	Enterococcus columbae	Enterococcus faecalis	Discordance
bacteria 2	Streptococcus pyogenes	Streptococcus anginosus	Discordance
bacteria 3	Streptococcus constellatus	Streptococcus agalactiae	Discordance
bacteria 4	Enterococcus faecalis	Enterococcus faecalis	Concordance
bacteria 5	Enterococcus faecalis	Enterococcus faecalis	Concordance
bacteria 6	Enterococcus durans	Enterococcus faecium	Discordance
bacteria 7	Enterococcus faecalis	Enterococcus faecalis	Concordance
bacteria 8	Enterococcus durans	Enterococcus faecium	Discordance
bacteria 9	Streptococcus agalactiae	Streptococcus agalactiae	Concordance
bacteria 10	Enterococcus faecium	Enterococcus faecium	Concordance
bacteria 11	Enterococcus faecalis	Enterococcus faecalis	Concordance
bacteria 12	Enterococcus faecium	Enterococcus faecium	Concordance
bacteria 13	Enterococcus faecalis	Enterococcus faecalis	Concordance
bacteria 14	Enterococcus faecium	Enterococcus faecium	Concordance
bacteria 15	Enterococcus casseliflavus	Streptococcus uberis	Discordance
bacteria 16	Enterococcus casseliflavus	Enterococcus faecalis	Discordance
bacteria 17	Enterococcus casseliflavus	Enterococcus faecium	Discordance
bacteria 18	Enterococcus casseliflavus	Enterococcus faecium	Discordance
bacteria 19	Enterococcus faecium	Enterococcus faecium	Concordance
bacteria 20	Enterococcus faecalis	Enterococcus faecalis	Concordance
bacteria 21	Enterococcus faecalis	Enterococcus faecium	Discordance
bacteria 22	Enterococcus faecalis	Enterococcus faecium	Discordance
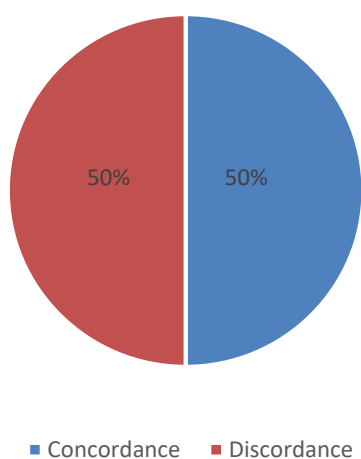
Figure 1: Concordance and discordance of Bis-Plus-D et Api 20 Strep results

Advantages and Disadvantages of using Api 20 Strep and Bis-Plus-D

The advantages and disadvantages of using Api 20 Strep and Bis-Pos-D are detailed in (table 2).

Discussion

Bis-Plus-D and Api 20 Strep systems are both bacterial identification systems. In this study, we identified 22 bacteria using Bis-Plus-D and Api 20 Strep. The 22 bacteria had the bacteriological characteristics of streptococci and enterococci, i.e. Gram-positive and catalase-negative cocci.

Concerning the results, half of the bacterial identification results were discordant between Bis-Plus-D and Api 20 Strep. Thus, it is necessary to analyze which of the 2 tests is the most reliable.

Regarding the bacterial identification qualities, they were all excellent (100%) for Api 20 Strep versus 36.4% for Bis-Plus-D. We can already see from this result that the Api 20 Strep test is more reliable than the Bis-Plus-D test. Similarly, looking at the probability scores of the 2 tests, those of the API 20 E are higher and some of these scores even reach 100%. Thus, all the results of this study showed that API 20 E is the best test for the identification of streptococci and enterococci. Nevertheless, it cannot be concluded that Bis-Plus-D is unreliable. Indeed, the identification process consists of recognizing an unknown bacterium by defining its membership in a species. It is based on the choice of a set of biochemical tests carried out by specific software. This set constitutes an identification kit. Depending on the response profile observed, a probability of belonging to each species is calculated. We retain the most probable species. A probability score between 60 and 100% means that the
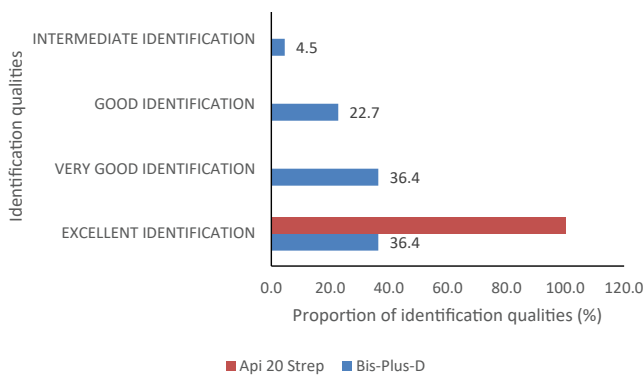
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Figure 2: Bacterial Identification qualities of Bis-Plus-D and Api 20 Strep
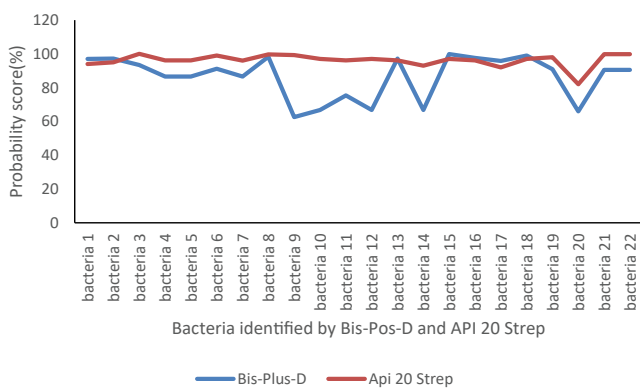

Figure 3: Probability scores for exact identification of bacteria by Bis-Plus-D and Api 20 Strep

Table 2: Advantages and disadvantages of using api 20 Strep and Bis-Plus-D

	Advantages	Disadvantages
Bis-Plus-D	Lasting expiration date	
	The price is cheaper in the trade	Need for several additional tests
	Identifies of all Gram-positive cocci, corynebacteria and anaerobes	Sometimes probability score less than 80%
Api 20 Strep	No need for additional tests	Short expiration date
	Probability score greater than 90%	The price is more expensive in the trade
		Cannot identifies all Gram positive cocci but only the streptococci
		Cannot identifies corynebacteria and anaerobes

confidence level is high and therefore the identification is reliable. For a score <60% the level of confidence is low, the software then proposing between two and four identifications [4-7]. In this study, all Bis-Plus-D probability scores were above 60% showing that this test is also reliable.

Furthermore, even though this study found that Api 20 Strep is more reliable than Bis-Plus-D, there are some disadvantages of using the Api 20 Strep. First, the expiry date of the kits does not exceed one year when they arrive at the laboratory. Second, the kit can only identify streptococci. Third, Third, Api 20E costs more expensive than Bis-Plus-D [8]. On the other hand, the use of Bis-Plus-D has many advantages. First, the kit has an expiration date of two to three years when it arrives at the laboratory. Secondly, the kit can identify all Gram-positive cocci, corynebacteria and anaerobes without exception. Third, the kit costs less than the API kits [9].

At the end of this study, we can conclude that the API 20 Strep test is the best test in the identification of streptococci and enterococci because its bacterial identification qualities are all excellent and its probability scores for exact identification of bacteria sometimes reach 100%.

Thus, the results of this study allow us to make some recommendations. First, Cypress Diagnostics should review the reagents contained in the Bis-Plus-D kit if there are any reagents that need to be added or changed to increase the reliability of the results. Secondly, large city laboratories that receive a lot of bacteriological analyzes should use the Api 20 Strep bacterial identification system as they can purchase it annually and the rapid expiry of this test is not a problem for them as well as the cost of the test. On the other hand, it is preferable that the small laboratories which do not do a lot of bacteriological analyze use the Bis-Plus-D because its expiry date is long, and its price is cheaper on the market. Likewise, the small laboratory does not need to buy other bacterial identification systems for the identification of other bacilli (corynebacteria and anaerobes) because the Bis-Plus-D is sufficient to identify them all.

In brief, the proper observance of standard operating procedures for each type of bacterial identification system should be carried out for performs a good bacterial identification in order to obtain the best probability score which is 100%.

Conclusion

Api 20 Strep remains the best identification bacterial system. However, after a review of the kit by Cypress Diagnostics, Bis-Plus-D can be used by medical analysis laboratories, especially if these laboratories do not need to perform a lot of bacterial identification tests. The proper observance of standard operating procedures for each type of bacterial identification system should be carried out for performs a good bacterial identification in order to obtain the best probability score which is 100%.

Acknowledgements

Competing interests

Authors have declared that no competing interests exist.

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