COMPARATIVE STUDY BETWEEN THE VITEK2 AND THE API 20 STEP REGARDING THE IDENTIFICATION OF MEDICALLY RELEVANT *STREPTOCOCCI.*

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Received: 30/12/2023
Accepted: 17/03/2024

Online ISSN: 2735-3540

ABSTRACT:

Background and aim of study: *The current study aims to compare between the Vitek2 GP ID BioMérieux® cards and the API 20 Strep BioMérieux® regarding the identification of medically relevant Streptococci.*

Methods: *This was a Cross sectional descriptive study that took place at the Central Microbiology Laboratory, Clinical Pathology Department, Ain Shams University Hospitals from August 2021 to February 2022. A Total 50 Medically relevant isolates of Streptococci recovered from different clinical samples were subjected to species identification using The API 20 Strep and the VITEK2 system using GP ID card.*

Results: *A statistically excellent agreement (%) = 45/50 = 90%; Disagreement (%) = 5/50= 10% was observed between VITEK® 2 system and the API 20 Strep method regarding identification results of the Streptococci spp. with (Kappa = 0.90 and p-value <0.001).*

Conclusions: *VITEK® 2 shows a very good and trustable accuracy and has dominate advantages in identification time as compared to manual API microsystems in clinical lab.*

Keywords: *Streptococci, Vitek2, API 20 Strep.*

INTRODUCTION:

Streptococci are gram-positive bacteria which are incriminated in many infections including pharyngitis, pneumonia, wound and skin infection, sepsis and endocarditis [(1)], On 5% sheep blood agar, colonies of Streptococci exhibit various degree of haemolysis which can be used as an early step in identifying clinical isolates [(2)].

Accurate identification of the organism is important to determine the pathogenic potential of species and track trends in antimicrobial susceptibility and infections [(3)]. Currently, no recognized method exists that is able to identify the organism to the species level satisfactorily, which has led to an inadequate reporting and potentially has serious effects on diagnosis and treatment [(4)].

Identification of Streptococcal species is usually performed using phenotypic methods such as API 20 Strep, VITEK 2 system and genotypic methods including analysis of the nucleotide of soda gene, encoding a manganese-dependent superoxide dismutase enzyme (Mn-SOD) [(4)], analysis of restriction patterns of the total chromosome (pulsed-field gel electrophoresis) or within specific genes (restriction fragment length polymorphism) (RFLP) of ribosomal RNA encoding genes. More recently, a multi-locus sequence analysis (MLSA) technique has been used for identification based on the nucleotide sequence analysis of seven housekeeping genes [(5&6)].

The analytical profile index Microsystems is commercial branded microbiology strip tests made and presented

by BioMérieux® in 1970. Each strip has 20 cupules containing dehydrated substrates that enable group or species identification of clinically important Streptococci and Enterococci based on their enzymatic activity or fermentation of sugars [7]. API Microsystems are known as trustable test strips used for microbiology studies. The API 20 Strep system is widely used and is generally accepted as a reliable identification system. However, the API is not capable of accepting new species from the recent phylogenetically led classifications which phenotypic distinctiveness may have made little contribution to the recognition of the new taxa [8].

Automated microbial identification systems e.g., the VITEK System (bioMérieux Inc.) decrease identification time, increase throughput of samples and make these within a highly automated, good and flexible piece of laboratory equipment [9&10]. VITEK 2 is an instrument used for automated phenotypic identification of medically relevant bacteria and yeasts (bioMérieux, Marcy l' Etoile, France). The new GP (gram positive) card consists of 43 biochemical tests that are monitored up to 8h. Many investigations have been conducted on VITEK®2 and they provided that it gives reliable results [11].

AIM OF THE STUDY:

Compare between the Vitek2 GP ID cards and the API® rapid ID 20 strep regarding the identification of medically relevant Streptococci.

MATERIALS AND METHODS:

Study design:

Cross-sectional descriptive study.

Study settings:

This study took place at the Central Microbiology Laboratory, Clinical Pathology Department, Ain-Shams University Hospitals from August 2021 to February 2022.

Sample size:

50 isolates of Streptococci recovered from variable clinical samples submitted to the Central Microbiology Lab, Clinical Pathology Department, Ain-Shams University Hospitals for routine culture and sensitivity.

Inclusion criteria:

Medically relevant isolates of Streptococci recovered from variable clinical samples, submitted to the Central Microbiology Lab, Clinical Pathology Department, Ain-Shams University Hospitals for routine culture and sensitivity.

Exclusion criteria:

Duplicate isolates from the same patient will be excluded.

Statistical analysis

The Comparison between groups with qualitative data was done by using Chi-square test and Monte Carlo correction: Correction for chi-square when more than 20% of the cells have expected count less than 5. Kappas over 0.75 is excellent, 0.40 to 0.75 is fair to good, and below 0.40 is poor.

Categorical agreement:

The results of both tests were compared, and categorical agreement (CA) was calculated as follows:

CA= number of agreed upon results/total number of results x100.

Ethical consideration:

This study was approved by the Faculty of Medicine, Ain-Shams University Research Ethics Committee (FMASU MS 451/2021).

RESULTS:

The isolates in our study were identified by conventional microbiological techniques including colonial morphology, gram stain characteristics and biochemical reactions. They were Enterococci (n=14), Strept pneumoniae (n=11), Viridans group Strept (n=16), Strept pyogenes (n=6), Strept

agalactiae (n=2) and Strept dysagalactiae (n=1) as shown in Table (1).

Table 1: Distribution of streptococci included in the study.

No	Streptococcus species	Quantity	Percentage
1	Enterococci	14	28%
2	Strept pneumoniae	11	22%
3	Viridans group Strept	16	32%
4	Strept pyogenes	6	12%
5	Strept agalactiae	2	4%
6	Strept dysagalactiae	1	2%
	Total	50	100%

Using the Vitek2 GP ID cards, in the current work, revealed that 16/50 (32%) were Viridans group Streptococci, 13/50 (26%) were Enterococci, 9/50 (18%) were Strept pneumoniae, 5/50 (10%) were Strept pyogenes, 2/50 (4%) were Strept agalactiae, 2/50 (4%) were Unidentified, 1/50 (2%) was Strept anginosus and 1/50 (2%) was kocuria rosea as shown in Figure (1).

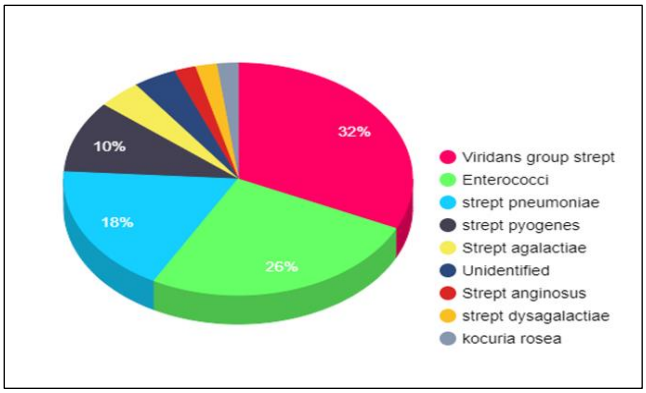


Figure 1: Results of the Vitek2 GP ID cards identification

On the other hand, identification using the API revealed that 15/50 (30%) were Viridans group Streptococci, 14/50 (28%) were Enterococci, 9/50 (18%) were Strept pneumoniae, 6/50 (12%) were Strept pyogenes, 2/50 (4%) were Strept agalactiae, 2/50 (4%) were Strept galloticus, 1/50 (2%) was Strept procinus and 1/50 (2%) was Strept dysagalctiae as shown in Figure (2).

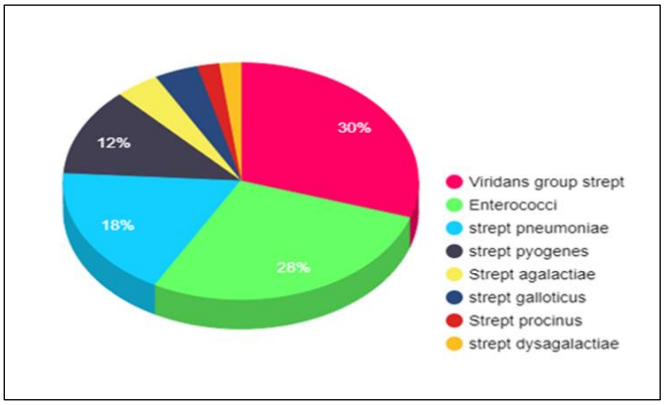


Figure 2: Results of identification using the API

The categorical agreement between both methods was 90% since 45 out of the 50 studied isolates had the same identification by both methods. Mismatched results were obtained in five isolates (5/50,10%). The Vitek2 failed to identify two isolates. Table(2)

Table 2: Shows categorical agreement between Vitek2 and API according to each Streptococcus species.

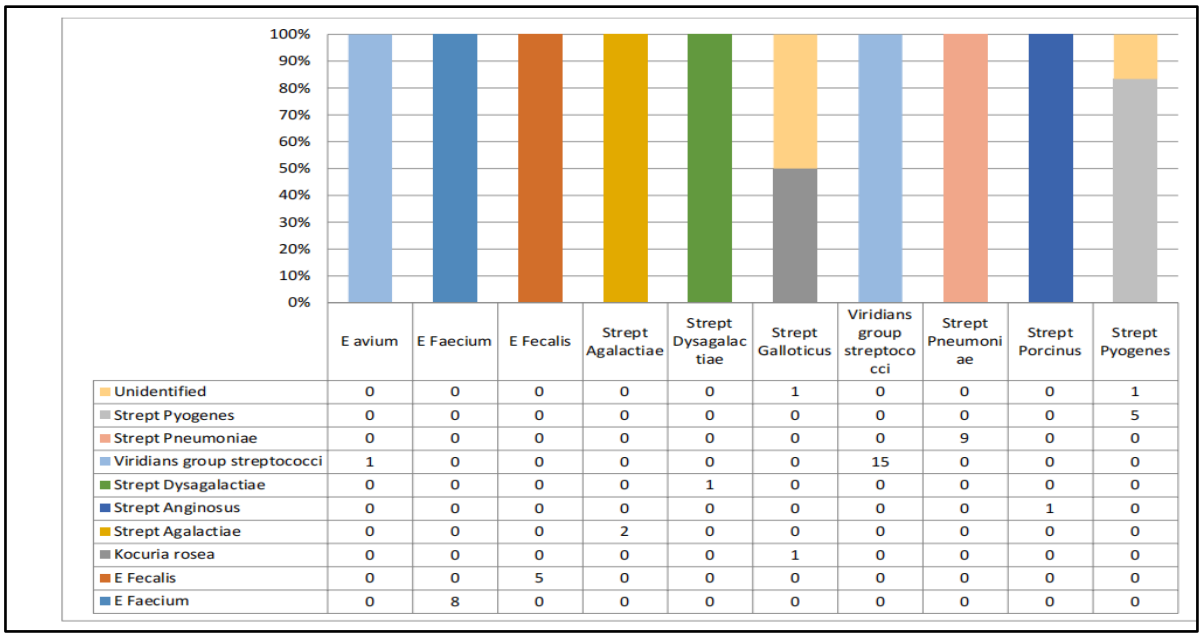
Isolate	No of isolates	Vitek2 Identification	API Identification	Agreement
Enterococci	14	13	13	13/14(92.8%)
Strep pneumoniae	11	9	9	9/11(81.8%)
Viridans group Streptococci	16	16	15	15/16(93.7%)
Strep pyogenes	6	5	6	5/6(83.3%)
Strep agalactiae	2	2	2	2/2(100%)
Strep dysagalactiae	1	1	1	1/1(100%)

A statistically excellent agreement (Kappa = 0.90, P<0.001) was observed between the results the Vitek2 GP ID cards and the API methods regarding the identification of the *Streptococci* spp. Table (3) and Figure (3).

Table 3: Agreement study between Vitek 2 Vs API:

			Vitek										Total
			E Faecium	E Fecalis	Kocuria rosea	Strept Agalactiae	Strept Anginosus	Strept Dysagalactiae	Viridians group streptococci	Strept Pneumoniae	Strept Pyogenes	Unidentified	
API	E avium	No.	0	0	0	0	0	0	1	0	0	0	1
		%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6.3%	0.0%	0.0%	0.0%	2.0%
	E Faecium	No.	8	0	0	0	0	0	0	0	0	0	8
		%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	16.0%
	E Fecalis	No.	0	5	0	0	0	0	0	0	0	0	5
		%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	10.0%
	Strept Agalactiae	No.	0	0	0	2	0	0	0	0	0	0	2
		%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.0%
	Strept Dysagalactiae	No.	0	0	0	0	0	1	0	0	0	0	1
		%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	2.0%
	Strept Galloticus	No.	0	0	1	0	0	0	0	0	0	1	2
		%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	50.0%	4.0%
	Viridians group streptococci	No.	0	0	0	0	0	0	15	0	0	0	15
		%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	93.8%	0.0%	0.0%	0.0%	30.0%
	Strept Pneumoniae	No.	0	0	0	0	0	0	0	9	0	0	9
		%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	18.0%
	Strept Porcinus	No.	0	0	0	0	1	0	0	0	0	0	1
		%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.0%
	Strept Pyogenes	No.	0	0	0	0	0	0	0	0	5	1	6
		%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	50.0%	12.0%
Total		No.	8	5	1	2	1	1	16	9	5	2	50
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Chi-square test			**317.621**										
p-value			**<0.001** (is highly significant association)**										

Measure of agreement	Value
Kappa agreement	0.90
p-value	<0.001



	E avium	E Faecium	E Fecalis	Strept Agalactiae	Strept Dysagalactiae	Strept Galloticus	Viridians group streptococci	Strept Pneumoniae	Strept Porcinus	Strept Pyogenes
Unidentified	0	0	0	0	0	1	0	0	0	1
Strept Pyogenes	0	0	0	0	0	0	0	0	0	5
Strept Pneumoniae	0	0	0	0	0	0	0	9	0	0
Viridians group streptococci	1	0	0	0	0	0	15	0	0	0
Strept Dysagalactiae	0	0	0	0	1	0	0	0	0	0
Strept Anginosus	0	0	0	0	0	0	0	0	1	0
Strept Agalactiae	0	0	0	2	0	0	0	0	0	0
Kocuria rosea	0	0	0	0	0	1	0	0	0	0
E Fecalis	0	0	5	0	0	0	0	0	0	0
E Faecium	0	8	0	0	0	0	0	0	0	0

Figure 3: Agreement between Vitek2 and API

DISCUSSION:

Streptococci are Gram-positive, non-spore forming, catalase negative spherical bacteria that occur in pairs which have broad significance in medicine. Streptococci are important ecologically as part of the normal microbial flora of humans. Some of the medically important Streptococci are Strep agalactiae, an etiologic agent of neonatal disease; E. faecalis and the viridans Streptococci are a major cause of endocarditis. Important members of the Viridans Streptococci include Strep mutans and Strep sanguis (involved in dental caries) and Strep mitis associated with bacteraemia, meningitis and pneumonia [12].

Accuracy in identification of the organism is important to determine the pathogenic potential of individual species and track trends in antimicrobial susceptibility and infections [3].

50 Streptococcal isolates were included in our study, 72% of the isolates (36/50) were recovered from clinical samples and 28% (14/50) were selected from proficiency test isolates, this was in accordance with *Teles C., et al.* [4], who analysed A panel of 42 isolates, 23 (54.8%) clinical isolates were obtained from infective endocarditis, blood and body fluid cultures, and For evaluation of the accuracy of the identification showed by each method the remaining 19 (45.2%) isolates were reference strains (ATCC).

In a comparison of the Vitek2 and the API 20 Strep identification results, there was 90% agreement (45/50 isolates) for all isolates tested. It was noticed that Vitek2 failed to identify 2 isolates and 3 isolates were mismatched between the two methods.

In our study, 11 pneumococcal isolates were tested. 9 of them were correctly identified by both API 20 Strep and Vitek 2C ID cards and the agreement between both methods was (81.8%). Our results were slightly lower than *Abele-Horn et al.* [13], who found that among 162 pneumococcal isolates, 150 isolates were correctly identified by both API 20 Strep and Vitek 2C ID cards with agreement (92.6%) between both methods. This may be due to the large number of isolates in the study. Also results obtained from a study done by *Ligozzi et al.* [14] declared that among 66 pneumococcal isolates, 64 isolates were correctly identified

by both methods with agreement (96.4%).

Among 14 clinical isolates of Enterococci included in our study, 13 isolates were correctly identified to the group level and one isolate was mismatched between both methods with agreement (92.8%). This came in accordance with a study by *Garcia et al.*[15] who studied 150 clinical isolates of Enterococci. Among those isolates, 131 were identified to the species level with both the VITEK 2 system and the API 20 Strep system with agreement (87%) between both methods.

On species level, 8 Enterococcus Faecium and 5 Enterococcus Faecalis were tested in our study. All of them were correctly identified by both methods with high level of confidence with agreement (100%) and this was not in accordance with *Ligozzi et al.* [14] who examined isolates of Enterococcus species, 55 isolates of E. faecalis and 28 of E. faecium, reported 92.7% (51/55) of Enterococcus faecalis were correctly identified but a low rate of correct identifications was noted with *E. faecium* 71.4% (20/28). In this study most E. faecium isolates were misidentified as E. hirae or E. durans, and these discrepancies could not be resolved because simple tests were not available to differentiate between these species.

The results of our study revealed that both Vitek 2C and API 20 Strep could identify 2 isolates of Streptococcus agalactiae with 100% agreement, this was in accordance with *Ligozzi et al.* [14] who examined 29 clinical isolates of Streptococcus agalactiae and reported that 28 isolates were correctly identified by both methods with agreement (96.5%).

Results of our study regarding Viridans group Streptococci revealed that API 20 Strep and Vitek 2C allocated 10 isolates in the same group (Mitis group) with agreement 66.6%. This is in agreement with *Teles C., et al.* [4] who studied A panel of 42 isolates of Viridans group Streptococci isolates. The API® system and Vitek 2C allocated 32 isolates to the same group with agreement 76.2%. It was noticed that the results of all Strep oralis and Strep mitis were presented as Strep mitis/Strep oralis with a high level of confidence, so the Vitek 2C does not differentiate among these species. Both techniques are currently not satisfactory for species identification of Viridans group Streptococci strains.

Conclusion:

Streptococci are important primary pathogen. Timely identification, good clinical correlation and proper therapeutic intervention can lead to favourable results. Our study demonstrated that the identification of Streptococci by the Vitek 2C and the API 20 Strep showed 90% agreement with kappa value 0.9. Systems such as API 20 Strep and Vitek 2C are of a limited utility for identification of Mitis Streptococci. Further studies to be done with large number of isolates especially on Viridans group Streptococci strains.

Conflict of interest:

None

Funding:

None. Author funded.

Author contribution:

In this study all listed authors have made substantial contributions to all of the following:

- research design, or acquisition, analysis or interpretation of data.
- drafting the paper or revising it critically.
- approving the submitted version.

We also declare that no-one who qualifies for authorship has been excluded from the list of authors.

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مقارنة بين Vitek2 و API20 Strep لتحديد المكورات السبحيه المهمه طبيا

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قسم الباثولوجيا الإكلينيكية - كلية الطب جامعة عين شمس

الخلفية وهدف الدراسة: تهدف الدراسة الحالية إلى المقارنة بين بطاقات Vitek2 GP ID BioMérieux® و API® Rapid ID20 Strep BioMérieux® فيما يتعلق بتحديد المكورات العقدية ذات الصلة طبيا.

الطرق: كانت هذه دراسة وصفية مقطعية تم إجراؤها في المعمل المركزي للأحياء الدقيقة، قسم علم الأمراض السريري، بمستشفيات جامعة عين شمس خلال الفترة ما بين أغسطس 2021 وفبراير 2022. تم شفاء إجمالي 50 عزلة ذات صلة طبيًا من المكورات العقدية من مختلف السريرية تم إخضاع العينات لتحديد الأنواع باستخدام نظام API® Rapid ID20 Strep ونظام VITEK® 2 باستخدام بطاقة هوية GP.

النتائج: اتفاق ممتاز إحصائيا (%) = 50/45 = 90%؛ لوحظ وجود خلاف (%) = 50/5= 10% بين نظام VITEK® 2 وطريقة API® Rapid ID20 Strep فيما يتعلق بنتائج التعرف على المكورات العقدية Streptococci spp. مع (κ= 0.90 والقيمة p <0.001).

الاستنتاجات: يوفر VITEK® 2 كنظام آلي دقة جيدة جدًا وموثوقة ويتمتع بمزايا مهيمنة في وقت التعريف مقارنة بأنظمة API الدقيقة اليدوية في المختبر السريري.