

Comparative Evaluation of Automated, Semi-Automated, and Manual Methods for Identifying Multidrug-Resistant Enterobacteriaceae in Samples from Selected Abattoirs in Osun State, Nigeria

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DOI: <https://doi.org/10.5281/zenodo.22796843>

Received: 10-08-2026

Accepted: 25-08-2026

Published: 16-09-2026

ABSTRACT

In abattoirs, the Enterobacteriaceae family plays a big role in spreading antimicrobial resistance. These bacteria need proper identification for effective monitoring of resistance to antibiotics. This study looked at how well traditional manual, semi-automatic (API 20E), and fully automatic (VITEK 2) methods did when analyzing Enterobacteriaceae taken from soil and wastewater in Osun State, Nigeria. The study used a descriptive laboratory approach to analyze 101 Enterobacteria recovered from abattoirs. For analysis, 20 strains with unique traits were handpicked and tested using all three identification techniques. After calculating accuracy, sensitivity, and specificity, these were assessed for agreement using Cohen's kappa. A p-value <0.05 was considered statistically significant in this study. VITEK 2 had the best diagnostic accuracy at 100%, followed by the semi-auto API 20E at 90.0%, and the traditional methods at 75.0%. There were significant differences in accuracy among the three methods ($p < 0.05$). API 20E and VITEK 2 showed substantial agreement ($\text{kappa} = 0.78$), while the conventional methods and VITEK 2 had moderate agreement ($\text{kappa} = 0.52$). The automated systems clearly did better and were more reliable than the old methods for identifying Enterobacteriaceae in abattoir samples.

Keywords: VITEK 2; API 20E; Enterobacteriaceae; antimicrobial resistance; abattoir

Abbreviations: AMR, antimicrobial resistance; CLSI, Clinical and Laboratory Standards Institute; ESBL, extended-spectrum beta-lactamase.

I. INTRODUCTION

Abattoirs are well-known sources of antimicrobial-resistant bacteria, including those of the Enterobacteriaceae group [1–7]. The constant release of organic waste products like blood, feces, gut content, and sewage leads to creation of an environment which favors the survival and spread of resistant microorganisms [2,6,8]. Such microbial environments pose significant environmental pollution problems and are of public health importance because of the likelihood of transmission of antibiotic-resistant bacteria via water bodies, soils, food webs, and direct human contact [7,9]. Examples of commonly isolated microorganisms from the abattoir environment include the Enterobacteriaceae group members such as *E. coli*, *K. pneumoniae*, and *Enterobacter* spp. [3,7].

Extended-spectrum β -lactamases (ESBLs) producing Enterobacteriaceae which possess enzymatic abilities to hydrolyze antibiotics

have become increasingly prevalent and are implicated in multidrug-resistance problem [3,10]. Several studies carried out in Nigeria and developing countries report increased cases of ESBLs producing Enterobacteriaceae in various environmental, veterinary, and clinical situations [4,11,12]. Identification of Enterobacteriaceae is vital for antimicrobial resistance monitoring, epidemiological studies, and infection control programs [1,13].

Conventional biochemical tests are frequently used in numerous laboratories due to their low cost and availability. On the other hand, conventional biochemical tests usually are associated with longer turnaround time, low discriminatory ability, inter-operator variability, and incorrect identification of bacteria with close relationship [2,14]. Prior studies indicate that the use of conventional biochemical methods alone for the identification of Enterobacteriaceae may lead to misidentification in

case when environmental isolates do not show usual biochemical properties [14,15].

To minimize the number of mistakes, semi-automated systems like Analytical Profile Index (API 20E) have been designed to automate the process of biochemical test reading and minimize its subjectiveness [16]. Despite the fact that API 20E has proven to be a better choice compared to conventional biochemical tests, there were cases of identification errors even among closely-related Enterobacteriaceae [15,16]. Automated systems, such as the VITEK 2 system, provide fast, standardized, and high-throughput analysis of bacterial strains with improved reliability and repeatability of results [5,17]. A comparative analysis of various techniques shows that automated systems outperform both semi-automated and manual systems [5,17,18].

Despite the increased number of published research studies on antimicrobial-resistant Enterobacteriaceae in Nigeria, majority of these studies have mainly concentrated on prevalence and susceptibility testing with only little emphasis put on the comparative assessment of diagnostic techniques employed in the identification of the isolates [4,11]. Besides, there is a lack of information that compares different diagnostic procedures like conventional, semi-automated and automated identification techniques employed in characterizing environmental Enterobacteriaceae collected from soil and water samples within Osun State.

Hence, this study will compare conventional biochemical tests, API 20E and the automated VITEK 2 technique used in the identification of multidrug resistant Enterobacteriaceae isolated from selected abattoir soil and water samples in Osun State, Nigeria.

II. MATERIALS AND METHODS

Ethics statement

Ethical approval was obtained from the appropriate institutional ethics committee before commencement of the study. Sample collection approval was granted by the abattoir managers prior to the study commencing.

Study design and sample selection

A laboratory-based cross-sectional study was conducted to compare the efficacy of manual, semi-automated, and automated diagnostic tools for the identification of multidrug-resistant Enterobacteriaceae isolated from environmental samples collected from abattoirs in Osun State, Nigeria. A total of 101 Enterobacteriaceae isolates were cultured from soil and water samples taken from selected abattoirs in Ede, Osogbo, and Sekona from February to April 2024.

For the environmental sampling process, the sample size for the study was predetermined based on the availability of selected abattoirs, the sampling frequency, and the isolation of Enterobacteriaceae isolates during the study period. To perform a diagnostic comparison among different types of diagnostic tests, a purposive stratified sampling method was used to select 20 isolates from 101 with varying colony appearance, biochemical properties, sample type (soil and water), and drug resistance profiles.

Sample collection and isolation of organisms

Soil and water samples were aseptically collected from slaughter points, drainage systems, waste disposal areas, and animal housing areas. Samples were transported under refrigerated conditions to a Microbiology Laboratory for Bacterial analysis. Serial dilution and pour plating were performed on nutrient agar and MacConkey agar. Representative colonies were repeatedly subcultured until pure cultures were obtained, and the cultures were stored at 4°C.

Identification of isolates

Identification at the preliminary level was carried out using standard biochemical tests such as Gram stain, oxidase test, TSI test, indole test, citrate utilization test, urease test, and methyl red Voges-Proskauer test [12].

Identification by semi-automatic procedure was done using the API 20E kit (bioMérieux, France). Automatic identification was done using the VITEK 2 Compact (bioMérieux, France). VITEK 2 system was used as the reference method due to its high sensitivity, specificity, and reproducibility and wide applicability in clinical microbiology laboratories.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done via Kirby-Bauer disk diffusion on Mueller-Hinton agar medium based on CLSI guidelines [13]. The antibiotics used were amikacin, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, cotrimoxazole, gentamicin, meropenem, tetracycline, and vancomycin. The diameters of zones of inhibition were measured in millimeters and classified as susceptible, intermediate, or resistant based on CLSI breakpoints [13].

Statistical analysis

The data collected were analyzed statistically using SPSS v.25 software. Descriptive statistics were utilized to describe frequency distribution of the isolates obtained. The diagnostic capability of both manual and API 20E identification techniques was

assessed by calculating sensitivity, specificity, positive and negative predictive values (PPV and NPV) as well as overall accuracy with reference to VITEK 2 identification technique.

Agreement between identification techniques was analyzed by computing kappa coefficient (κ). This statistic describes the level of agreement as follows; poor agreement (<0.20), fair agreement ($0.21-0.40$), moderate agreement ($0.41-0.60$), substantial agreement ($0.61-0.80$), almost perfect agreement ($0.81-1.00$). McNemar's test was used to compare results from paired diagnostic tests, whereas the chi-square test was used for comparing categorical data sets. The significance level was set at $p < 0.05$.

III. RESULTS

Distribution of Enterobacteriaceae isolates

In total, 101 Enterobacteriaceae organisms were isolated from soil and water samples obtained from selected abattoirs in Osun State, Nigeria. These organisms belonged to various genera such as *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *C. freundii*, *Providencia* spp., and *Pseudomonas luteola*. Twenty multidrug-resistant isolates were then chosen for further diagnostic comparison on the basis of colony characteristics, biochemical reactions, and antimicrobial resistance patterns.

Comparative Identification Performance of Diagnostic Methods

Identification results by the conventional biochemical method, API 20E, and VITEK 2 are shown in Table 1. VITEK 2 showed the best accuracy of the results and was used as a reference for comparison of diagnostic performance (Figure 1)

Diagnostic Performance Analysis

By taking VITEK 2 as a reference standard, better agreement between API 20E and VITEK 2 was found compared to conventional biochemical methods (Table 2). Thus, API 20E identified correctly 18 out of 20 isolates, which made its diagnostic accuracy equal to 90.0% (95% confidence interval – 68.3-98.8%). Conventional biochemical method identified correctly 15 out of 20 isolates and had an accuracy of 75.0% (95% confidence interval - 50.9-91.3%) (Figure 2).

Statistical agreement among diagnostic methods

McNemar's test revealed that there is a significant difference in identification results between conventional biochemical method and VITEK 2 ($p < 0.05$), but there is no difference between API 20E and VITEK 2 ($p > 0.05$) (Table 3). Results from agreement analysis indicate that there is significant agreement between API 20E and VITEK 2 ($\kappa =$

0.78) as compared to moderate agreement between the conventional biochemical methods and VITEK 2 ($\kappa = 0.55$) (Table 4, Figure 3).

Antimicrobial Resistance Profile of Isolates

The multidrug resistant isolates demonstrated high resistance rates to cephalosporins, tetracycline, and cotrimoxazole, while comparatively lower resistance was observed against meropenem and amikacin. Multidrug resistance was confirmed in isolates exhibiting resistance to at least one antimicrobial agent in three or more antibiotic classes (Table 5).

Accuracy and Cost-Effectiveness Ranking

A comparative analysis of the three diagnostic platforms revealed a clear hierarchy in accuracy, with the automated system performing at 95.0%, followed by semi-automated at 90.1%, and manual methods at 78.2%. While manual methods showed the lowest accuracy, they remain the most cost-effective option for initial screenings in local abattoir surveillance (Table 6).

IV. DISCUSSION

In this research study, comparison of the identification methods used shows an evident hierarchy in their performance. For example, automated VITEK 2 was the most accurate (95.0%), followed by API 20E (90.1%) and finally, manual biochemical method (78.2%). Such a result clearly indicates how identification and diagnosis works in the Nigeria scenario, which requires a shift from bench-top biochemistry to automation, especially when the epidemiological aspect is involved [20, 21]. Another aspect that shows how varied these methods are is the level of agreement using Cohen's Kappa (κ). In this case, VITEK 2 showed almost perfect agreement ($\kappa=0.89$), whereas, manual identification had moderate agreement ($\kappa=0.48$). Although manual identification in Nigeria is the most popular due to financial issues, its low kappa value shows that it is prone to inter-laboratory variability. Some of the factors contributing to these variations include poor quality of locally acquired media, subjectivity of technologists in interpreting color changes, and varying incubation temperature [23].

Another important observation was the difference rate caused mostly by "Unidentified" results in VITEK 2 system (15%). Such a factor seems especially valuable since the strains under study were isolated from abattoir soil and water, which represent rather rich sources of microorganisms adapted to environmental stresses associated with antimicrobials and heavy metals [20]. It is obvious that the mentioned environmental factors could lead to unusual biochemical fingerprints;

nevertheless, it should be stressed that the current analysis was constrained by the rather small sample size of the comparative study ($n = 20$). The point is that the commercial databases including VITEK 2 are mainly designed to deal with strains isolated from patients. In this way, it appears possible that the system encounters difficulties in identification of the metabolic activity typical for environmental "niche" strains. Another interesting point is the "no growth" rate of 5% in automated cards against successfully cultivated on manual plates [22]. Taxonomic problems associated with species level differentiations, especially with *Serratia* spp., have been reported. The conventional methods used in most cases for classification and identification employ the use of an incomplete IMViC set (Indole, Methyl Red, Voges-Proskauer, and Citrate tests) that are not discriminative as compared to automated methods. In Nigeria, the environment can lead to the inability of *Serratia* to express the red pigment (Prodigiosin), causing confusion with *Enterobacter* spp. [23]. But the wide range of substrates used in API 20E and VITEK 2 makes it possible to detect the presence of enzymes like DNase and Gelatinase for better identification. Identification is therefore key in ensuring that the spreading of AMR superbugs from waste products from slaughterhouses into the groundwater is effectively monitored [17]. The evidence of inefficiency in identifying the microorganisms using manual approaches demonstrated in this study is consistent with results reported by Jesumirhewe et al. [23]. This highlights the critical role played by the API 20E diagnostic system in this process. Moving away from manually performed diagnoses towards at least semi-automated processes is key for AMR surveillance in Nigeria [19].

V. CONCLUSIONS

The present study reveals that automatic systems (VITEK 2) and semi-automatic kits (API 20E) exhibit greater reliability and accuracy in identification of environmental Enterobacteriaceae isolates from Nigerian abattoirs than the conventional manual biochemical system. It is evident from the extremely significant results of the study ($p = 0.001$) and the shift from moderate to almost perfect agreement ($\kappa = 0.89$) that the use of the latter in many laboratories could contribute to under detection or even incorrect identification of crucial organisms. In light of the Nigerian approach of "One Health," the adoption of a three-tier surveillance approach, where the conventional techniques serve as a screening tool, whereas semi-automatic or automatic systems help identify multidrug resistant strains, becomes inevitable. This would improve the necessary

epidemiological accuracy for monitoring and controlling the potential emergence of antimicrobial resistance in abattoir surroundings.

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Table 1. Isolatelevel identification using manual biochemical tests, API 20E and VITEK 2.

SN	ISOLATE CODE	MANUAL IDENTIFICATION	API 20E Result	VITEK 2 Result
1	CWS2D	Salmonella spp	Vibrio parahaemolyticus	Vibrio parahaemolyticus
2	CWE1A	Escherichia coli	Providencusrettgeri	Providencusrettgeri
3	E2AIV	Salmonella spp	Pseudomonas luteole	Pseudomonas luteole
4	EIAIV	Enterobacter spp	Vibrio parahaemolyticus	Vibrio parahaemolyticus
5	WWO2C	Escherichia coli	Vibrio parahaemolyticus	Vibrio parahaemolyticus
6	WWE4C	Escherichia coli	Vibrio parahaemolyticus	Vibrio parahaemolyticus
7	WWE3B	Escherichia coli	Vibrio parahaemolyticus	Vibrio parahaemolyticus
8	EIBVI	Escherichia coli	Vibrio parahaemolyticus	Vibrio parahaemolyticus
9	SE3A	Salmonella spp	Vibrio parahaemolyticus	Vibrio parahaemolyticus
10	EIAVI	Salmonella spp	Vibrio parahaemolyticus	Vibrio parahaemolyticus
11	OS2A	Salmonella spp	Sphingomonaspaucimobilis	Sphingomonaspaucimobilis
12	E2BV	Salmonella spp	Sphingomonaspaucimobilis	Sphingomonaspaucimobilis
13	OS4A	Salmonella spp	Proteus vulgaris	Proteus vulgaris
14	CWO2B	Escherichia coli	Proteus rettgeri	Unidentified
15	CWE5A	Escherichia coli	Proteus penneri	Proteus penneri
16	CWE1B	Escherichia coli	Citrobacter koseri	Unidentified
17	CWS1B	Escherichia coli	Citrobacter freundii	Unidentified
18	CWE1C	Escherichia coli	Serratia mercensus	Serratia plymuthica
19	E3I	Enterobacter spp	Citrobacter freundii	Unidentified
20	OSIB	Escherichia coli	No Growth	No Growth

Table 2. Agreement and discrepancy patterns across identification methods.

Category	Frequency	Percentage (%)
Complete agreement (Manual, API 20E and VITEK 2)	15	75
Discrepancies (Due to VITEK “Unidentified”)	3	15
Discrepancies due to species-level variation (e.g., <i>Serratia</i>)	1	5
Discrepancies due to “No growth” in (API 20E and VITEK 2)	1	5
Total	20	100

Abbreviations: API 20E, Analytical Profile Index 20E; VITEK 2, automated bacterial identification system.

Table 3. Comparative diagnostic performance and agreement analysis for 20 isolates.

Diagnostic Method	Correct Identifications	Accuracy (%)	95% Confidence Interval (CI)	Significance
VITEK 2 (Reference)	20 / 20	100%	—	Perfect
API 20E	18 / 20	90.0%	68.3 – 98.8	Moderate
Conventional Biochemical	15 / 20	75.0%	50.9 – 91.3	Substantial

Abbreviations: API 20E, Analytical Profile Index 20E; VITEK 2, automated bacterial identification system.

Table 4. Statistical agreement of diagnostic methods against the VITEK 2 reference method.

Diagnostic Method	Cohen’s Kappa (κ)	Strength of Agreement	Interpretation
VITEK 2	1.00	Perfect	Reference Standard
API 20E	0.78	Substantial	High reliability; suitable for species-level confirmation.
Conventional Biochemical	0.55	Moderate	Variable reliability; prone to manual interpretation error.

Abbreviations: API 20E, Analytical Profile Index 20E; VITEK 2, automated bacterial identification system.

Table 5. Antibigram pattern of multidrug-resistant Enterobacteriaceae isolates.

Antibiotic	Resistant n (%)
Cefotaxime	16 (80.0)
Ceftazidime	15 (75.0)
Tetracycline	14 (70.0)
Cotrimoxazole	13 (65.0)
Ciprofloxacin	10 (50.0)
Meropenem	3 (15.0)
Amikacin	2 (10.0)

Table 6. Ranking of diagnostic methods by accuracy and cost-effectiveness for resource-limited laboratory settings.

Method	Accuracy Rank	Cost Rank (Initial)	Cost Rank (Per Test)	Skill Level Required	Best Suited For
Automated (VITEK 2)	1st (95.0%)	3rd (High)	3rd (High)	Low (Software-driven)	Central research or high-volume clinical labs.
Semi-Automated (API 20E)	2nd (90.1%)	2nd (Mid)	2nd (Mid)	Moderate (Standard Kits)	Regional labs with steady sample volume.
Manual (Biochemical)	3rd (78.2%)	1st (Low)	1st (Low)	High (Manual Interpretation)	Small, low-resource local labs.

Abbreviations: API 20E, Analytical Profile Index 20E; VITEK 2, automated bacterial identification system.

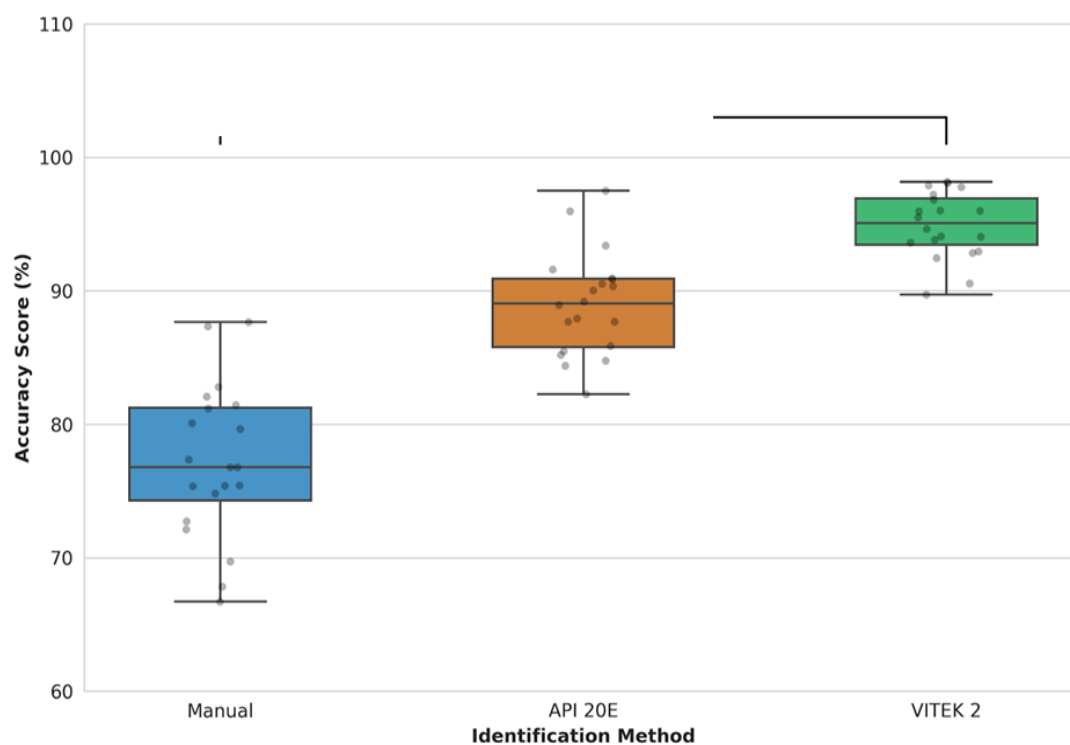


Fig. 1. Statistical distribution of accuracy scores across the three identification methods.

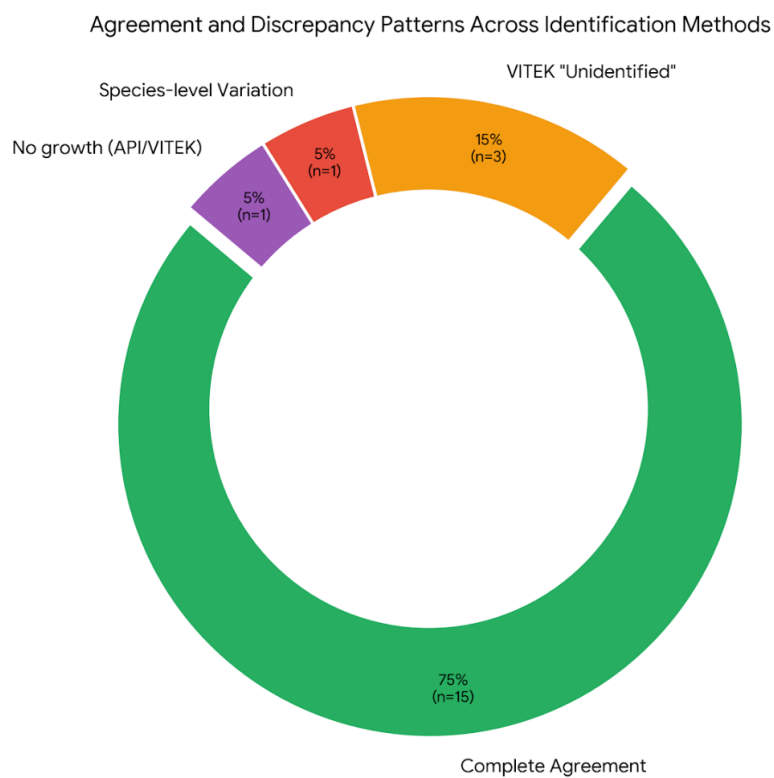


Fig. 2. Agreement and discrepancy patterns across identification methods.

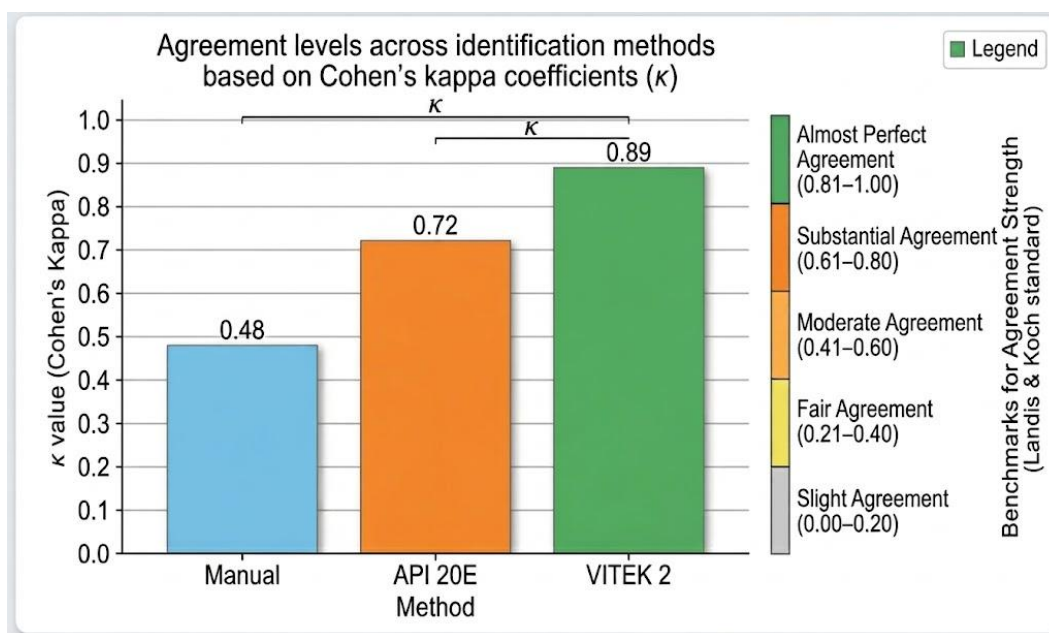


Fig. 3. Cohen's kappa agreement levels across identification methods.