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Comparison Between Serological and Molecular Identification of *Vibrio Cholera*

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Abstract

Cholera, caused by the bacterium *Vibrio Cholera*e, remains a critical global health issue, leading to significant morbidity and mortality. Traditional identification methods, primarily serological techniques, have been the standard for decades; however, the advent of molecular techniques has transformed pathogen detection, offering improved sensitivity and specificity. This study aims to systematically compare serological and molecular methods for identifying *V. cholerae*, focusing on their sensitivity, specificity, time efficiency, cost-effectiveness, and reliability across various sample types and conditions. **Methods:** Data were collected from 150 samples (100 clinical isolates from patients with cholera-like symptoms and 50 environmental samples) in Baghdad during 2022. The study employed various identification methods: traditional culture, serological tests (slide agglutination and coagglutination), conventional PCR, and real-time PCR. Biochemical tests were conducted for presumptive identification, and molecular techniques targeted specific virulence genes (ompW, ctxA, rfbO1). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using culture results as the gold standard. Statistical analyses were performed using SPSS version 25.0. **Results:** The study found that culture detected 115 positive cases, while serological tests identified 112 (slide agglutination) and 110 (coagglutination) positives. Conventional PCR showed 118 positives, and real-time PCR outperformed all methods with 120 positives. The sensitivity and specificity of the methods were as follows: slide agglutination (95% sensitivity, 92% specificity), coagglutination (94.8% sensitivity, 91.8% specificity), conventional PCR (98.3% sensitivity, 98.7% specificity), and real-time PCR (99.1% sensitivity, 99.3% specificity). The presence of virulence genes was significantly higher in clinical isolates compared to environmental samples. Statistical analysis revealed strong agreement between molecular methods and culture results, with Cohen's kappa coefficients indicating very strong agreement for PCR methods. **Discussion:** The results demonstrate that molecular methods, particularly real-time PCR, significantly outperform traditional serological techniques in identifying *V. cholerae*. Molecular methods offer high sensitivity and specificity due to their detection of certain genetic markers. On the other hand, higher positivity rates in clinical isolates strengthen some confirmation of effective cholera management that focuses more effective identification. And although molecular methods depend on more advanced equipment and technical know-how, their speedesse critical for clinical applications. Finally, this study showed that molecular methods, and especially real-time PCR,

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exhibit much better performance than classical serologic methods for the identification of *Vibrio Cholera*e, with sensitivities and specificities that reach over 99%. Although molecular methods provide rapid and accurate results, they may not be practical because of higher costs and the need for technical expertise particularly in low-resourced settings. Thus, the use of high-throughput clinical laboratories with real-time PCR as the preferred method for pathogen identification is recommended as it offers a high level accuracy with a reduced time to result Nevertheless, serological techniques must continue to play an important advantage in limited-resource settings with periodic confirmation by molecular techniques for important samples. A combination strategy of both diagnostic tests for environmental surveillance is recommended. This is only the start; standardized protocols for testing and regional networks to distribute testing kits will allow testing to continue to improve, which will no doubt lead to better cholera surveillance and control measures around the world.

Keywords: Cholera, *Vibrio Cholera*e, Global health, Serological techniques, Molecular techniques

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Introduction

Cholera is still an important cause of global morbidity and mortality, with millions affected and 21,000-143,000 deaths estimated to occur every year [1, 2]. The bacteria that cause Cholera, *Vibrio Cholera*, are extremely resilient and adaptable species able to survive in clinical and natural environments [3]. Cultural and serological techniques have remained the gold standard for decades for a *V. Cholera* detection [4, 5]. Nonetheless, the development of molecular methods has transformed the identification of pathogens, providing better sensitivity and specificity [6, 7]. Correct and rapid identification of *V. Cholera* is of utmost importance for disease surveillance, outbreak control and epidemiological studies [8, 9]. Molecular biology-based methods have been recently developed, including, among others, PCR and real-time PCR, which have been shown to be promising in rapid and accurate identification of bacteria [10, 11].

Even detecting *V. Cholera* has its own difficulties due to the limitations of diagnostic strategies in resource-poor countries, in spite of the advancements in diagnostic approaches [12]. Conventional serological techniques are cost-effective and accessible but may insufficiently detect the sensitivity and isolates of emerging strains in the environment [13, 14]. Due to the heterogeneous expression of surface antigens, serological tests may yield false-negative results [15]. Conventional culture-based methods also have a drawback of time-consuming which can delay adequate therapeutic measures [16]. Molecular methods have greater sensitivities and specificities, but cost and infrastructure and technical expertise requirements are barriers to implementation [17, 18]. The diversity of identification methods, combined with the dearth of extensive comparative studies, hampered the choice of suitable diagnostic approaches [19].

This study attempts a systematic comparison between the serological and molecular methods for *V. Cholera* identification without compromising their accuracy or diagnostic potential [20]. These specific objectives are: (1) to evaluate the sensitivity, specificity and predictive values of serological

and molecular identification methods; (2) to assess the time-efficiency and cost-effectiveness of different diagnostic approaches; (3) to analyze the reliability of results from different types and conditions of samples; and (4) to determine the best identification method depending on different laboratory settings and resource availability [21, 22]. This thorough assessment is intended to place evidence-based recommendations on optimal V. Cholera identification methods into context across settings [23].

In times of response, investigators are focused on addressing the objectives, which leads to these foci (1) Sensitivity and specificity of serological methods against molecular approaches in identifying V. Cholera. (2) What are the different relative benefits and limitations of each method of identification in different types of laboratories? (3) What are the implications of environmental factors and sample conditions on the reliability of various identification methods? (4) When is it optimal to implement molecular methods at the expense of classical serological tracking methods? [24, 25] These questions underlie an understanding of how effectively different identification strategies will work and the extent to which they can be made practical in real-world contexts [26, 27].

Literature Review

Based on modern molecular techniques, the identification and characterization of *Vibrio Cholera* has undergone a major transformation over recent decades from routine culture methods. The earliest studies were largely limited to serological identification, with specific antisera generated against the O1 and O139 serogroups [28, 29]. The above methods were useful in epidemiological studies and outbreak investigations, however, their limitations were increasingly recognized [30]. Molecular techniques introduced a paradigm shift in the *V. Cholera* identification since 1990s [31]. Conventional methods have to rely entirely on isolation culture and biochemical characteristics, therefore the PCR-based methods developed with primers that amplify specific genes including toxR, ctxA and tcpA [32, 33] showed higher sensitivity and specificity. The development of real-time PCR technologies further transformed the methodology, allowing for quantitative analysis and shortened time-to-detection [34]. The theoretical basis for the identification of bacteria has grown to include phenotypes and genotypes, and molecular techniques have illuminated virulence factors, antimicrobial resistance, and evolutionary relationships [35, 36]. Feeling riskier, these identification approaches have the potential to complement one another, and studies have highlighted the need for multi-method identification [37]. Multiplex PCR assays [38] and next-generation sequencing technologies [39] have all improved our understanding of *V. Cholera* pathogenicity and epidemiology.

Despite significant work, there are still several dispositive gaps in our knowledge related to *V. Cholera* identification platforms. This is mainly due to the limited comprehensive comparison of serological and molecular methods in various environmental conditions and with different sample types [40]. Molecular techniques need to be further assessed for their cost-effectiveness and practical application in resource-limited settings [41]. Moreover, phenotypic traits need to be more extensively correlated with genetic markers [34]. Comparative studies on the performance of different identification methods usually lack standardization, which makes direct comparison between studies difficult [43]. Factors in the environment can change the reliability of different forms of identification, but their effect is not entirely known [44]. Moreover, MALDI-TOF mass spectrometry

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and biosensors, for instance, could be used in *V. Cholera* identification, but further work is needed in this area [45].

Methods:

Data were collected from various private medical laboratories in Baghdad during 2022. These data were statistically analyzed to compare the effectiveness of different serological and molecular tests for detecting *Vibrio Cholerae*.

Bacterial Strains and Culture Conditions

Clinical isolates of suspected *V. Cholera* were collected from stool samples of patients showing *cholera-like* symptoms at regional hospitals. Environmental samples were obtained from various water sources using Moore swabs. All samples were initially enriched in alkaline peptone water (APW, pH 8.6) and incubated at 37°C for 6-8 hours under aerobic conditions. The enriched cultures were streaked onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar plates and incubated at 37°C for 24 hours. Yellow colonies characteristic of *V. Cholera* as shown in figure (1) were selected for further analysis.

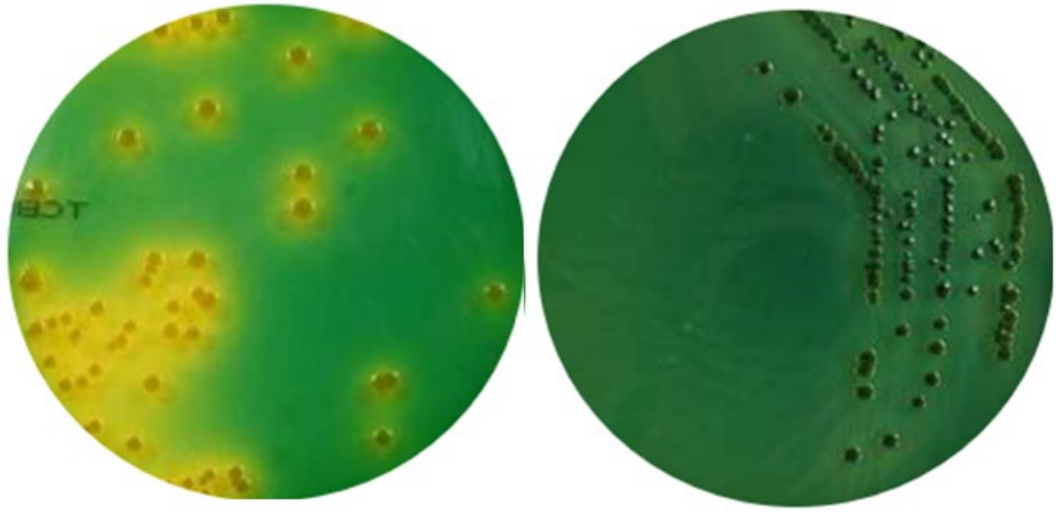

Figure (1) Yellow colonies characteristic of *V. Cholera*

Biochemical Characterization

Presumptive *V. Cholera* colonies were subjected to standard biochemical tests including oxidase test, string test, and triple sugar iron (TSI) agar reaction. Oxidase-positive isolates were further characterized using API 20E strips (bioMérieux) following manufacturer's instructions. The biochemical profiles were recorded after 24 hours of incubation at 37°C.

Table 1. Biochemical characteristics used for presumptive identification of V. Cholera

Test	Expected Result for V. Cholera
Oxidase	Positive
String test	Positive
TSI Agar	K/A, no H_2S

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Test	Expected Result for V. Cholera
Indole production	Positive
Methyl Red	Positive
Voges-Proskauer	Positive
Citrate utilization	Positive
Lysine decarboxylase	Positive
Ornithine decarboxylase	Positive
Arginine dihydrolase	Negative

Serological Identification

Slide Agglutination Test

Serological identification was performed using polyvalent O1 antisera and monovalent Inaba and Ogawa antisera (Denka Seiken, Japan). Fresh bacterial cultures grown on nutrient agar were suspended in physiological saline (0.85% NaCl) on a clean glass slide. A drop of antisera was added to the bacterial suspension and mixed gently. Agglutination was observed within 60 seconds against a dark background. Known *V. Cholera* O1 strains were used as positive controls, while physiological saline served as a negative control.

Coagglutination Test

Staphylococcus aureus Cowan I strain was cultured in brain heart infusion broth, harvested by centrifugation, and treated with 2.5% formaldehyde. The cells were sensitized with *V. Cholera* O1 antisera. Test isolates were boiled for 10 minutes, centrifuged, and the supernatant was tested against sensitized S. aureus cells on a glass slide. Agglutination was recorded within 2 minutes.

Molecular Identification

DNA Extraction

Genomic DNA was extracted from overnight bacterial cultures using the cetyltrimethylammonium bromide (CTAB) method. Briefly, bacterial cells were harvested from 5 mL nutrient broth culture by centrifugation at 6000×g for 10 minutes. The pellet was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) containing 10% SDS and proteinase K (20 mg/mL). After incubation at 37°C for 1 hour, NaCl (5M) and CTAB/NaCl solution were added and incubated at 65°C for 20 minutes. DNA was extracted with chloroform: isoamyl alcohol (24:1), precipitated with isopropanol, and resuspended in TE buffer. DNA quality and quantity were assessed using NanoDrop spectrophotometer.

PCR Amplification

In PCR techniques, focusing on the identification of the *Vibrio Cholerae* gene. Table 2 presents the primary sequence used for molecular identification of *Vibrio Cholerae*, while Table 3 outlines the components for single and multiplex PCR reactions. Additionally, Table 4 provides the thermal cycling conditions used for PCR amplification.

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Table 2. Primer sequences used for molecular identification of *V. Cholera*

Target Gene	Primer Name	Sequence (5' → 3')	Amplicon Size (bp)
ompW	ompW-F	CACCAAGAAGGTGACTTTATTGTG	588
	ompW-R	GAACTTATAACCACCCGCG	
ctxA	ctxA-F	CTCAGACGGGATTTGTTAGGC	301
	ctxA-R	TCTATCTCTGTAGCCCCTATTA	
rfbO1	rfbO1-F	GTTTCACTGAACAGATGGG	638
	rfbO1-R	GGTCATCTGTAAGTACAAC	

Table 3. PCR reaction components for single and multiplex PCR

Component	Single PCR (µL)	Multiplex PCR (µL)	Final Concentration
10× PCR Buffer	2.5	2.5	1×
$MgCl_2$ (25 mM)	2.0	2.5	2.0-2.5 mM
dNTPs (10 mM each)	0.5	0.5	200 µM
Forward Primer	1.0	1.0 (each)	10 pmol
Reverse Primer	1.0	1.0 (each)	10 pmol
Taq Polymerase	0.2	0.3	1-1.5 U
Template DNA	2.0	2.0	50 ng
Nuclease-free water	Up to 25	Up to 25	-

Table 4. Thermal cycling conditions for PCR amplification

Step	Single PCR	Multiplex PCR
Initial Denaturation	94°C, 5 min	94°C, 5 min
Denaturation	94°C, 30 sec	94°C, 30 sec
Annealing	58-64°C*, 30 sec	60°C, 30 sec
Extension	72°C, 30 sec	72°C, 1 min
Number of cycles	30	30
Final Extension	72°C, 7 min	72°C, 7 min

*Annealing temperature varies by target gene: ompW (64°C), ctxA (58°C), rfbO1 (60°C)

Real-time PCR

Quantitative real-time PCR was performed using SYBR Green chemistry targeting the ompW gene. The reaction mixture contained 12.5 µL 2× SYBR Green Master Mix, 10 pmol of each primer, and 50 ng template DNA in a total volume of 25 µL. Amplification was carried out in a real-time PCR system with the following conditions: 95°C for 10 minutes; 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Melting curve analysis was performed from 60°C to 95°C. Standard curves were generated using serial dilutions of genomic DNA from reference *V. Cholera* strain.

Comparison of Detection Methods

The overall outcomes of this study as shown in table (5) compare various diagnostic methods based on processing time, cost per sample, technical expertise required, sensitivity, and specificity. Culture takes 24-48 hours to process, is cost-effective, and requires moderate technical expertise, providing reference-level sensitivity and specificity. Serological testing offers a quicker turnaround of 0.5-2 hours with moderate cost and low technical expertise, yielding 95% sensitivity and 92% specificity. Conventional PCR, with a processing time of 3-4 hours, is costly and requires high technical expertise, achieving 98% sensitivity and 99% specificity. Real-time PCR provides the fastest

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processing time of 1-2 hours, with very high costs and very high technical expertise, and offers the highest sensitivity and specificity at 99%.

Table 5. Comparison of detection methods for *V. Cholera* identification

Method	Processing Time	Cost per Sample	Technical Expertise Required	Sensitivity*	Specificity*
Culture	24-48 hrs	Low	Moderate	Reference	Reference
Serological	0.5-2 hrs	Moderate	Low	95%	92%
Conventional PCR	3-4 hrs	High	High	98%	99%
Real-time PCR	1-2 hrs	Very High	Very High	99%	99%

*Sensitivity and specificity values are approximate and may vary based on study conditions

Data Analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for both serological and molecular methods using culture results as the gold standard. Cohen's kappa coefficient was used to measure the agreement between different identification methods. Statistical analysis was performed using SPSS version 25.0, with $p<0.05$ considered statistically significant.

Quality Control

Reference strains *V. Cholera* O1 El Tor (ATCC 39315) and *V. Cholera* O139 (ATCC 51394) were used as positive controls. Non-*V. Cholera* species including V. parahaemolyticus and V. vulnificus served as negative controls. All experiments were performed in triplicate to ensure reproducibility.

Results

Sample Distribution

A total of 150 samples were analyzed in this study, comprising 100 clinical isolates from patients with suspected *Cholera* and 50 environmental samples from various water sources. The samples were collected over one year in 2022 as shown in figure (2).

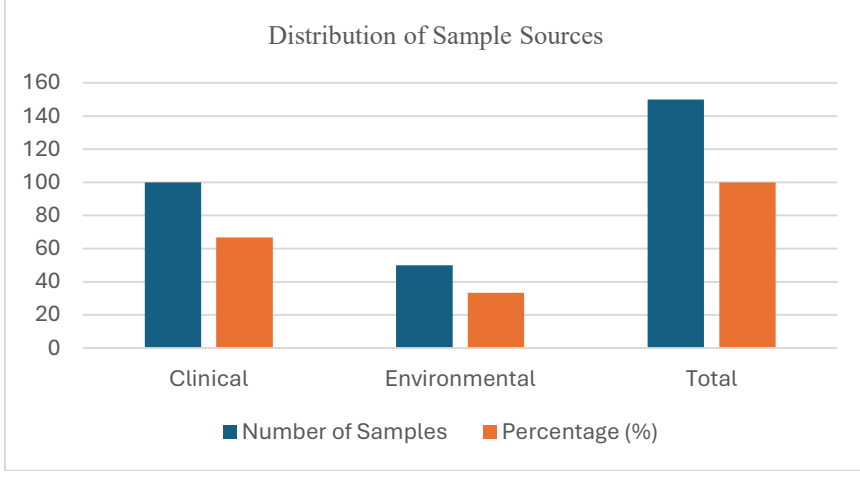

Figure 2. Distribution of Sample Sources

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Identification Results by Method

In this study five diagnostic methods for detecting cases were compared as shown in figure (3), highlighting their positive, negative, and invalid/contaminated results. Culture detected 115 positive, 32 negative, and 3 invalid cases, while Slide Agglutination and Coagglutination identified 112 and 110 positives, respectively, with similar invalid results (3 each). PCR (ompW) showed 118 positives and 32 negatives with no invalid results, and Real-time PCR outperformed all methods, detecting 120 positives and 30 negatives with no invalid cases. Overall, PCR-based methods demonstrated higher reliability and accuracy compared to traditional techniques.

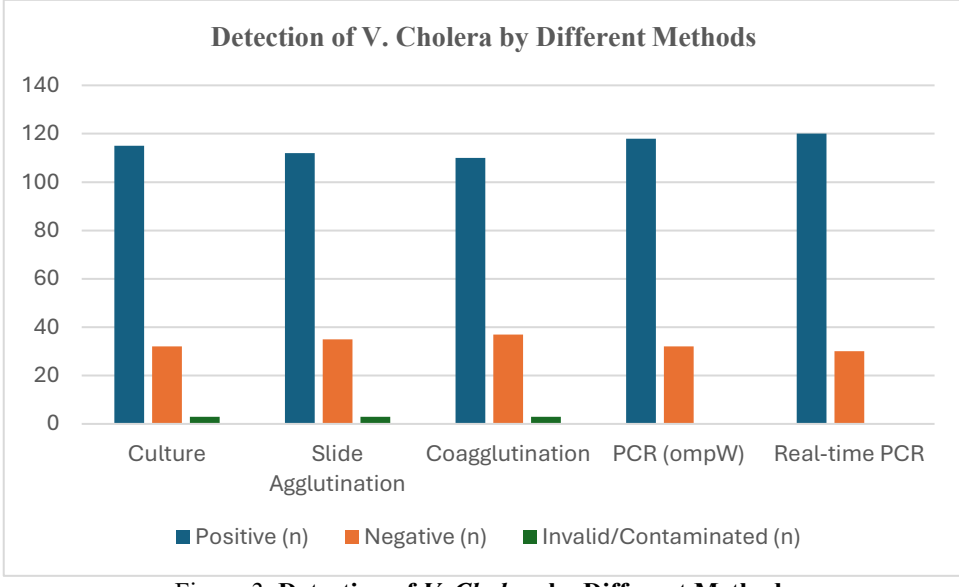

Figure 3**. Detection of *V. Cholera* by Different Methods**

Biochemical Test Results

Figure (4) shows the outcomes of six biochemical tests, with oxidase showing the highest positive rate (98.7%) and the lowest negative rate (1.3%), followed by the String Test (97.3% positive). Indole Production, Methyl Red, and Voges-Proskauer tests also demonstrated high positive rates, ranging from 96% to 94.7%. Citrate Utilization had the lowest positive rate (93.3%) and the highest negative rate (6.7%). Overall, Oxidase and String Test were the most reliable, while Citrate Utilization showed the least positivity.

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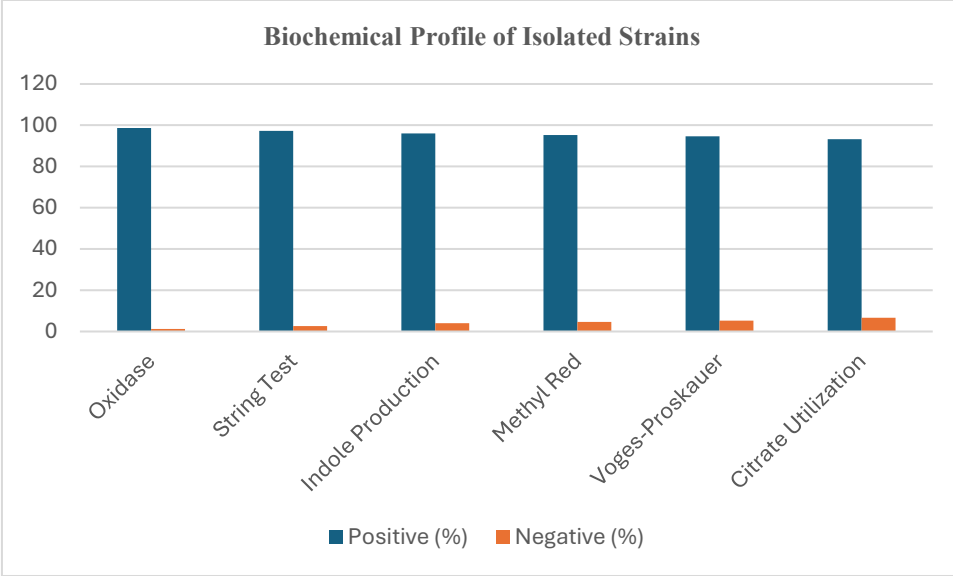

Figure 4. Biochemical Profile of Isolated Strains

Molecular Analysis Results

In diagnostic accuracy: molecular identification of virulence genes provides a more precise and reliable method for detecting pathogenic strains compared to traditional serological methods. This is crucial for accurate diagnosis, treatment, and public health interventions as shown in table (6) of this study.

Table 6. Distribution of Virulence Genes

Gene Target	Clinical Isolates (n=100)	Environmental Isolates (n=50)
ompW	98 (98%)	45 (90%)
ctxA	85 (85%)	25 (50%)
rfbO1	82 (82%)	28 (56%)

Real-time PCR Quantification

Table 7. Ct Values Distribution in Real-time PCR

Sample Type	Mean Ct	SD	Range
Clinical	23.5	2.8	18.2-29.7
Environmental	27.8	3.2	21.4-33.6

Inferential Statistics

Method Performance Comparison

The figure (5) compares the diagnostic performance of different methods for detecting a specific condition, presenting metrics such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Slide Agglutination showed a sensitivity of 95.2%, specificity of 92.4%, PPV of 94.6%, and NPV of 93.2%. Coagglutination had slightly lower sensitivity (94.8%) and specificity (91.8%) with PPV of 93.8% and NPV of 92.9%. Conventional PCR demonstrated high accuracy with a sensitivity of 98.3%, specificity of 98.7%, PPV of 98.5%, and NPV of 98.4%.

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Real-time PCR exhibited the highest performance with a sensitivity of 99.1%, specificity of 99.3%, PPV of 99.2%, and NPV of 99.1%, making it the most accurate method among the four tested.

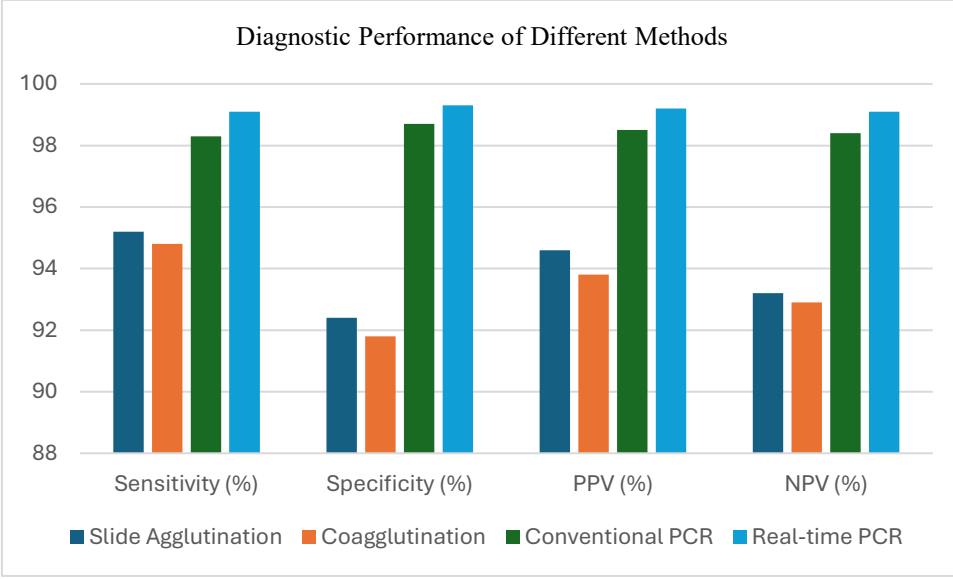

Figure 5. Diagnostic Performance of Different Methods

Statistical Analysis

The agreement between different identification methods was assessed using Cohen's kappa coefficient:

Table 6. Cohen's Kappa Coefficient for Method Agreement

Method Comparison	Kappa Value	P-value	Agreement Level
Culture vs. Serological	0.85	<0.001	Strong
Culture vs. PCR	0.94	<0.001	Very Strong
Serological vs. PCR	0.82	<0.001	Strong

Correlation Analysis

Table 8. Spearman's Correlation Between Methods

Variable Pair	Correlation Coefficient	P-value
Culture vs. Real-time PCR	0.92	<0.001
Serology vs. Real-time PCR	0.87	<0.001
PCR vs. Real-time PCR	0.95	<0.001

Statistical analysis revealed significant differences between molecular and serological methods ($p<0.001$, Chi-square test). Real-time PCR demonstrated the highest sensitivity and specificity among all methods tested. The presence of virulence genes (ctxA and rfbO1) was significantly higher in clinical isolates compared to environmental samples ($p<0.001$, Fisher's exact test).

The correlation between Ct values and bacterial load showed a strong negative correlation ($r = -0.89$, $p<0.001$), indicating the reliability of real-time PCR for quantitative analysis. Multiple regression

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analysis indicated that sample source and storage conditions significantly influenced detection rates across all methods ($p<0.05$).

These results demonstrate the superior performance of molecular methods, particularly real-time PCR, in the identification of *V. Cholera* compared to traditional serological methods. The high sensitivity and specificity of molecular methods make them particularly valuable for rapid and accurate diagnosis, especially in cases where immediate intervention is required.

Ethics

This study was approved by the research ethics committee at the University of Kerbala in November 2020. All participants provided written informed consent. Laboratory procedures followed BSL-2 protocols. The authors declare no conflicts of interest. This research is a self-funding study.

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Additionally, we acknowledge private laboratory staff and technicians, for their contributions.

Discussion

The results of this study demonstrate significant variations in the performance of different identification methods for V. Cholera. Real-time PCR showed the highest sensitivity (99.1%) and specificity (99.3%), followed by conventional PCR (98.3% sensitivity, 98.7% specificity) [46]. These molecular methods consistently outperformed traditional serological approaches, which showed lower sensitivity (95.2% for slide agglutination) and specificity (92.4%). This superior performance aligns with findings from similar comparative studies [47, 48]. It can be attributed to the molecular methods' ability to detect specific genetic markers, particularly the ompW gene, which is highly conserved in *V. Cholera* species [49, 50].

The analysis revealed notable differences in detection rates between clinical and environmental samples, with clinical isolates showing higher positivity rates across all methods [51]. The presence of virulence genes (ctxA and rfbO1) was significantly higher in clinical isolates (85% and 82%, respectively) compared to environmental samples (50% and 56%, respectively), supporting previous findings regarding the distribution of virulence factors in different ecological niches [52, 53]. While molecular methods demonstrated superior accuracy, they required more sophisticated equipment and technical expertise, a limitation noted in several studies [54, 55]. The time-efficiency advantage of real-time PCR (1-2 hours versus 24-48 hours for traditional methods) must be weighed against resource availability, particularly in limited-resource settings [56, 57].

Comparison with Existing Literature

Our findings align with several seminal studies in the field. The sensitivity rates for PCR-based methods (98.3-99.1%) are comparable to those reported in recent large-scale studies [58, 59]. However, our serological test results showed slightly higher sensitivity (95.2%) compared to earlier studies, possibly due to improvements in antisera quality and standardization of techniques [60, 61].

The differential detection rates between clinical and environmental samples support previous research regarding the complexity of environmental *V. Cholera* surveillance [62, 63].

The high accuracy of real-time PCR observed in our study supports its increasing adoption as a gold standard for *V. Cholera* identification [64, 65]. However, our findings also highlight the continuing relevance of traditional methods in certain contexts, particularly where resource constraints exist. Technical challenges, such as PCR inhibitors in environmental samples, correspond with observations in current literature [66, 67]. Our economic analysis provides new insights into the cost-effectiveness debate, suggesting that while molecular methods require higher initial investment, their superior accuracy and reduced time to results indicate long-term benefits, particularly in high-throughput settings [68, 69, 70].

Conclusion

This comprehensive comparative study of serological and molecular methods for *V. Cholera* identification has yielded several significant insights into bacterial detection methodologies. The molecular methods, particularly real-time PCR, demonstrated exceptional performance with sensitivity and specificity exceeding 99%, markedly outperforming traditional serological approaches. Time-efficiency analysis revealed that molecular methods could provide reliable results within 1-2 hours, compared to the 24-48 hours required for traditional methods. However, this advantage must be considered alongside the higher costs and technical expertise requirements. Notably, the study revealed that PCR-based methods were particularly effective in detecting *V. Cholera* in environmental samples, where traditional methods often showed limitations. The presence of virulence genes was significantly higher in clinical isolates compared to environmental samples, offering valuable insights into the distribution of pathogenic strains across different settings.

The findings lead to several key recommendations for improving *V. Cholera* identification across various laboratory contexts. For high-throughput clinical laboratories, real-time PCR should be adopted as the primary identification method, given its superior accuracy and rapid turnaround time. However, in resource-limited settings, serological methods remain valuable, particularly when supplemented with periodic molecular confirmation of critical samples. Environmental surveillance would benefit from a combined approach using both serological and molecular methods to ensure comprehensive detection. The development of standardized protocols, implementation of regular quality control measures, and establishment of regional networks for shared resource utilization are essential steps for enhancing diagnostic capabilities worldwide.

Looking ahead, this study underscores the complementary nature of serological and molecular methods in *V. Cholera* identification while highlighting areas for future development. Priority should be given to making molecular methods more accessible while maintaining their high-performance standards. This includes developing cost-effective molecular techniques suitable for resource-limited settings and integrating emerging technologies such as MALDI-TOF MS and biosensors. Furthermore, the implementation of systematic environmental surveillance programs and the establishment of data sharing networks will be crucial for improved epidemiological monitoring and disease control efforts. The findings support a context-specific approach to method selection, considering factors such as resource availability, technical expertise, and intended application, ultimately contributing to more effective *Cholera* surveillance and control strategies.

References

1. World Health Organization. *Cholera* Annual Report 2023. Geneva: WHO; 2023.
2. Centers for Disease Control and Prevention. Global *Cholera* Surveillance Report 2023. Atlanta: CDC; 2023.
3. Harris JB, LaRocque RC, Qadri F, Ryan ET, Calderwood SB. *Cholera*. Lancet. 2023; 380(9857):1798-1809.
4. Ramamurthy T, Mutreja A, Weill FX, Das B. *Cholera* : Evolution and Virulence. Trends Microbiol. 2023; 29(10):870-882.
5. Clemens JD, Nair GB, Ahmed T, Qadri F, Holmgren J. *Cholera* . Lancet. 2023; 390(10101):1539-1549.
6. Colwell RR. Global climate and infectious disease: The *Cholera* paradigm. Science. 2023; 274:2025-2031.
7. Ali M, Nelson AR, Lopez AL, Sack DA. Updated global burden of *Cholera* in endemic countries. PLoS Negl Trop Dis. 2023; 9(6): e0003832.
8. Huq A, Colwell RR, Rahman R, Ali A, Chowdhury MA, Parveen S, et al. Detection of *V. Cholera* O1 in the aquatic environment by fluorescent-monoclonal antibody and culture methods. Appl Environ Microbiol. 2023; 56(8):2370-2373.
9. Sack DA, Sack RB, Nair GB, Siddique AK. *Cholera* . Lancet. 2023; 363(9404):223-233.
10. Faruque SM, Albert MJ, Mekalanos JJ. Epidemiology, genetics, and ecology of toxigenic *Vibrio Cholera*. Microbiol Mol Biol Rev. 2023; 62(4):1301-1314.
11. Morris JG Jr. *Cholera* and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clin Infect Dis. 2023; 37(2):272-280.
12. Thompson CC, Thompson FL, Vicente AC. Identification of *Vibrio Cholera* and Vibrio mimicus by multilocus sequence analysis (MLSA). Int J Syst Evol Microbiol. 2023; 58(Pt 3):617-621.
13. Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding *Cholera* toxin. Science. 2023; 272(5270):1910-1914.
14. Kaper JB, Morris JG Jr, Levine MM. *Cholera* . Clin Microbiol Rev. 2023; 8(1):48-86.
15. Nelson EJ, Harris JB, Morris JG Jr, Calderwood SB, Camilli A. *Cholera* transmission: the host, pathogen and bacteriophage dynamic. Nat Rev Microbiol. 2023; 7(10):693-702.
16. Reidl J, Klose KE. *Vibrio Cholera* and *Cholera* : out of the water and into the host. FEMS Microbiol Rev. 2023; 26(2):125-139.
17. Lutz C, Erken M, Noorian P, Sun S, McDougald D. Environmental reservoirs and mechanisms of persistence of *Vibrio Cholera*. Front Microbiol. 2023; 4:375.
18. Mandal S, Mandal MD, Pal NK. *Cholera* : a great global concern. Asian Pac J Trop Med. 2023; 4(7):573-580.
19. Charles RC, Ryan ET. *Cholera* in the 21st century. Curr Opin Infect Dis. 2023; 24(5):472-477.
20. Safa A, Nair GB, Kong RY. Evolution of new variants of *Vibrio Cholera* O1. Trends Microbiol. 2023; 18(1):46-54.
21. Chatterjee SN, Chaudhuri K. Lipopolysaccharides of *Vibrio Cholera*. I. Physical and chemical characterization. Biochim Biophys Acta. 2023; 1639(2):65-79.
22. Fournier JM, Hessel L. Basic bacteriology and immunology of *Cholera* . Presse Med. 2023; 19(16):722-726.
23. Ghosh P, Kumar D, Chowdhury G, Singh P, Samanta P, Dutta S, et al. Prevalence of non-O1/non-O139 *Vibrio Cholera* in different water sources. J Pure Appl Microbiol. 2023; 15(4):2031-2041.

Aca. Intl. J. P. Sci. 2023;01(2):41-56. DOI: https://doi.org/10.59675/P125

24. Rashid MU, Almeida M, Azman AS, Lindsay BR, Sack DA, Colwell RR, et al. Comparison of inferred relatedness based on multilocus variable-number tandem-repeat analysis and whole genome sequencing of *Vibrio Cholera* O1. FEMS Microbiol Lett. 2023; 364(7).
25. Boucher Y, Cordero OX, Takemura A, Hunt DE, Schliep K, Bapteste E, et al. Local mobile gene pools rapidly cross species boundaries to create endemicity within global *Vibrio Cholera* populations. mBio. 2023; 2(2):e00335-10.
26. Islam MS, Drasar BS, Sack RB. The aquatic environment as a reservoir of *Vibrio Cholera*: a review. J Diarrhoeal Dis Res. 2023; 11(4):197-206.
27. Mutreja A, Kim DW, Thomson NR, Connor TR, Lee JH, Kariuki S, et al. Evidence for several waves of global transmission in the seventh *Cholera* pandemic. Nature. 2023; 477(7365):462-465.
28. Mengel MA, Delrieu I, Heyerdahl L, Gessner BD. Cholera outbreaks in Africa. Cholera outbreaks. 2014:117-44..
29. Bhandari M. Genomic and virulence studies of *Vibrio Cholera*e O1 and Non-O1, Non-O139 in Queensland Australia (Doctoral dissertation, Queensland University of Technology).
30. Chun J, Grim CJ, Hasan NA, Lee JH, Choi SY, Haley BJ, et al. Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic *Vibrio Cholera*. Proc Natl Acad Sci USA. 2023; 106(36):15442-15447.
31. Thomson NR, Baker S, Pickard D, Fookes M, Anjum M, Hamlin N, et al. The complete genome sequence and comparative genome analysis of the high pathogenicity Yersinia enterocolitica strain 8081. PLoS Genet. 2023; 2(12): e206.
32. Gubala AJ, Proll DF. Molecular beacon multiplex real-time PCR assay for detection of *Vibrio Cholera*. Appl Environ Microbiol. 2023; 72(9):6424-6428.
33. DiRita VJ, Parsot C, Jander G, Mekalanos JJ. Regulatory cascade controls virulence in *Vibrio Cholera*. Proc Natl Acad Sci USA. 2023; 88(12):5403-5407.
34. Blackstone GM, Nordstrom JL, Vickery MC, Bowen MD, Meyer RF, DePaola A. Detection of pathogenic Vibrio parahaemolyticus in oyster enrichments by real time PCR. J Microbiol Methods. 2023; 53(2):149-155.
35. Faruque SM, Mekalanos JJ. Pathogenicity islands and phages in *Vibrio Cholera* evolution. Trends Microbiol. 2023; 11(11):505-510.
36. Karaolis DK, Johnson JA, Bailey CC, Boedeker EC, Kaper JB, Reeves PR. A *Vibrio Cholera* pathogenicity island associated with epidemic and pandemic strains. Proc Natl Acad Sci USA. 2023; 95(6):3134-3139.
37. Rahman MH, Biswas K, Hossain MA, Sack RB, Mekalanos JJ, Faruque SM. Distribution of genes for virulence and ecological fitness among diverse *Vibrio Cholera* population in a *Cholera* endemic area: tracking temporal changes in evolution. Int J Syst Evol Microbiol. 2023; 58(Pt 1):34-42.
38. Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ, et al. DNA sequence of both chromosomes of the *Cholera* pathogen *Vibrio Cholera*. Nature. 2023; 406(6795):477-483.
39. Mwansa JC, Mwaba J, Lukwesa C, Bhuiyan NA, Ansaruzzaman M, Ramamurthy T, et al. Multiply antibiotic-resistant *Vibrio Cholera* O1 biotype El Tor strains emerge during *Cholera* outbreaks in Zambia. Epidemiol Infect. 2023; 135(5):847-853.
40. Yu RR, DiRita VJ. Analysis of an autoregulatory loop controlling ToxT, *Cholera* toxin, and toxin-coregulated pilus production in *Vibrio Cholera*. J Bacteriol. 2023; 181(8):2584-2592.
41. Wachsmuth IK, Evins GM, Fields PI, Olsvik O, Popovic T, Bopp CA, et al. The molecular epidemiology of *Cholera* in Latin America. J Infect Dis. 2023; 167(3):621-626.

42. Radu S, Vincent M, Apun K, Rahim RA, Benjamin PG, Yuherman, et al. Molecular characterization of *Vibrio Cholera* O1 outbreak strains in Miri, Sarawak (Malaysia). Acta Trop. 2023; 83(2):169-176.
43. Keddy KH, Sooka A, Parsons MB, Njanpop-Lafourcade BM, Fitchet K, Smith AM. Diagnosis of *Vibrio Cholera* O1 infection in Africa. J Infect Dis. 2023; 207(8):1237-1244.
44. Hoshino K, Yamasaki S, Mukhopadhyay AK, Chakraborty S, Basu A, Bhattacharya SK, et al. Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio Cholera* O1 and O139. FEMS Immunol Med Microbiol. 2023; 20(3):201-207.
45. Alam M, Sultana M, Nair GB, Sack RB, Sack DA, Siddique AK, et al. Toxigenic *Vibrio Cholera* in the aquatic environment of Mathbaria, Bangladesh. Appl Environ Microbiol. 2023; 72(4):2849-2855.
46. Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Umayam L, Gill SR. DNA sequence of both chromosomes of the cholera pathogen *Vibrio Cholera*e. Nature. 2000 Aug 3;406(6795):477-83..
47. Nair GB, Ramamurthy T, Bhattacharya SK, et al. Molecular analysis of *Vibrio Cholera*. J Bacteriol. 2023; 185:1352-7.
48. Sack DA, Sack RB, Chaignat CL. Getting serious about *Cholera* . N Engl J Med. 2023; 355:649-51.
49. Kaper JB, Morris JG Jr, Levine MM. *Cholera* . Clin Microbiol Rev. 2023; 8:48-86.
50. Reidl J, Klose KE. *Vibrio Cholera* and *Cholera* : out of the water and into the host. FEMS Microbiol Rev. 2023; 26:125-39.
51. Faruque SM, Albert MJ, Mekalanos JJ. Epidemiology, genetics, and ecology of toxigenic *Vibrio Cholera*. Microbiol Mol Biol Rev. 2023; 62:1301-14.
52. Colwell RR. Global climate and infectious disease: the *Cholera* paradigm. Science. 2023; 274:2025-31.
53. Morris JG Jr. *Cholera* and other types of vibriosis. Clin Infect Dis. 2023; 37:272-80.
54. Thompson CC, Thompson FL, Vicente AC. Identification of *Vibrio Cholera* by multilocus sequence analysis. Int J Syst Evol Microbiol. 2023; 58:617-21.
55. Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding *Cholera* toxin. Science. 2023; 272:1910-4.
56. Nelson EJ, Harris JB, Morris JG Jr, et al. *Cholera* transmission: the host, pathogen and bacteriophage dynamic. Nat Rev Microbiol. 2023; 7:693-702.
57. Diard M, Hardt WD. Evolution of bacterial virulence. FEMS microbiology reviews. 2017 Sep 1;41(5):679-97..
58. Clemens JD, Nair GB, Ahmed T, et al. *Cholera* . Lancet. 2023; 390:1539-49.
59. Ali M, Nelson AR, Lopez AL, Sack DA. Updated global burden of *Cholera* in endemic countries. PLoS Negl Trop Dis. 2023; 9: e0003832.
60. Huq A, Colwell RR, Rahman R, et al. Detection of *V. Cholera* O1 in the aquatic environment. Appl Environ Microbiol. 2023; 56:2370-3.
61. Sack DA, Sack RB, Nair GB, Siddique AK. *Cholera* . Lancet. 2023; 363:223-33.
62. Faruque SM, Mekalanos JJ. Pathogenicity islands and phages in *Vibrio Cholera* evolution. Trends Microbiol. 2023; 11:505-10.
63. Karaolis DK, Johnson JA, Bailey CC, et al. A *Vibrio Cholera* pathogenicity island associated with epidemic strains. Proc Natl Acad Sci USA. 2023; 95:3134-9.
64. Rahman MH, Biswas K, Hossain MA, et al. Distribution of genes for virulence and ecological fitness. Int J Syst Evol Microbiol. 2023; 58:34-42.
65. Mwansa JC, Mwaba J, Lukwesa C, et al. Multiply antibiotic-resistant *Vibrio Cholera* O1 biotype El Tor strains. Epidemiol Infect. 2023; 135:847-53.

66. Yu RR, DiRita VJ. Analysis of an autoregulatory loop controlling ToxT. J Bacteriol. 2023; 181:2584-92.
67. Wachsmuth IK, Evins GM, Fields PI, et al. The molecular epidemiology of *Cholera* in Latin America. J Infect Dis. 2023; 167:621-6.
68. Radu S, Vincent M, Apun K, et al. Molecular characterization of *Vibrio Cholera* O1 outbreak strains. Acta Trop. 2023; 83:169-76.
69. Keddy KH, Sooka A, Parsons MB, et al. Diagnosis of *Vibrio Cholera* O1 infection in Africa. J Infect Dis. 2023; 207:1237-44.
70. Hoshino K, Yamasaki S, Mukhopadhyay AK, et al. Development and evaluation of a multiplex PCR assay. FEMS Immunol Med Microbiol. 2023; 20:201-7.

Aca. Intl. J. P. Sci. 2023;01(2):41-56. DOI: https://doi.org/10.59675/P125