THE EFFECT OF USING MODERN TECHNOLOGY IN DETECTING AND EXAMINING INTESTINAL MICROBE

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ABSTRACT
Infectious gastroenteritis is a diagnosis that depends greatly on traditional culture, which has a slow turnaround time and is unable to detect viruses, parasites, or fastidious bacteria, thus creating a serious ethiological gap in knowledge. The goal of the study was to determine the impact of shotgun metagenomic next -generation sequencing (mNGS) on the detection and analysis of intestinal microbes comparatively to its diagnostic capability, rapidity, and overall yield against the standard of care. A prospective, lab-based study where 200 stool samples were subjected to a conventional culture and a standardized mNGS workflow and compared statistically in terms of sensitivity, specificity and turnaround time, was conducted on patients with acute gastroenteritis. Findings indicated that mNGS was far better than culture, having a sensitivity of 96.7 -1 (95 CI: 88.7-99.1-1) and a 33.2 -hour improvement in average time-to-result (35.2 vs. 68.4-1 hours, p = 0.001). Moreover, mNGS added significant diagnostic information to the other tests by detecting viral and parasitic pathogens in 29.4% of culture-negative samples and antimicrobial resistance genes in 44.5% of all samples. We conclude that contemporary metagenomic technology is a revolutionary, more holistic method of detecting intestinal pathogens with the potential to provide rapid, true, and comprehensive diagnosis which is essential in enhancing the management and antimicrobial stewardship of patients.
Keywords: Diagnostic Yield, Metagenomics, Microbiome, Pathogen Detection, Turnaround Time

INTRODUCTION

Infectious gastroenteritis remains one of the major health problems of people in the world that causes significant morbidity, mortality, and economic losses [1]. Identification of enteric pathogens is crucial and timely to achieve optimal clinical care, prevention, and antimicrobial stewardship. Traditionally, the diagnosis of bacterial gastrointestinal infections was conducted mainly according to the traditional culture-based methods [2]. Despite their fundamental

nature, these methods have some underlying drawbacks, such as the need for microorganisms to grow under artificially established conditions, which results in a lengthy turnaround time of 4872 hours, a loss of fastidious or non-culturable organisms, and the loss of viral and parasitic organisms that contribute a substantial proportion of gastroenteritis cases [4]. As a result, there is a persistent critical diagnostic loophole where numerous cases remain etiologically undetermined and may undermine patient care.

The area of this issue is wide, as both local and global populations are impacted. Globally, diarrhoeal diseases cause some of the highest numbers of deaths in children under five and are especially common in places with limited resources, although similar diseases are also a serious health concern in developed countries [5]. In our local setting, the same epidemiological trends are witnessed, where gastroenteritis is a common health-seeking behavior with healthcare consultations. The use of empirical antibiotics, which is deployed in situations of diagnostic uncertainty, is one of the factors that have led to the increasing crisis of antimicrobial resistance (AMR), to which modern medicine is a primary threat [6]. This highlights the importance of the urgency to have diagnostic paradigms that are faster, sensitive, and comprehensive enough to guide focused therapeutic interventions.

Microbial diagnostics in an era of high-throughput DNA sequencing technologies has arrived. Shotgun metagenomic next-generation sequencing (mNGS) is one of them, and it is a revolutionary tool [7]. Compared to specific molecular tests like PCR, mNGS provides a hypothesis-free, culture-independent technique that has the potential to discover and characterize all nucleic acids, bacteria, viruses, fungi, and parasites in a clinical sample at once [8]. The theory behind this technology is that it is able to overcome the cultivation biases of the traditional methods and offer a more holistic and quicker way of detecting the pathogen [9]. Moreover, mNGS offers the one and only opportunity to investigate functional properties of the microbial community, such as virulence factor and antimicrobial resistance gene profiling of the clinical sample directly, which consequently provides a lot more than just the taxonomic identification [10].

Although it has a theoretical potential, the mNGS implementation has yet to be introduced into clinical microbiology practice to detect intestinal pathogens, as a robust and methodical validation of the new technique compared to the current standard of care has been necessary [11]. There has been a research gap in terms of the direct and prospective comparison of a validated mNGS workflow to traditional culture in a clinical diagnostic laboratory real-world situation. There was no specific emphasis on strict statistical examination of performance measures, turnaround period, and value added [12]. The technical feasibility of mNGS had already been proven in previous studies, but its operational excellence and applicability in a working clinical setting needed clarification. There were some methodological questions about its sensitivity and specificity in a heterogeneous population of patients, the amount of time-to-diagnosis that it would reduce, and the clinical usefulness of its enhanced detection capacity, such as the interpretation of co-infections and AMR gene carriage [13].

It is based on this context that we carried out the current study. The main aim was to measure quantitatively the impact of introducing modern technology of mNGS on the detection and analysis of intestinal microbes [14]. Three particular research questions helped us in investigating the topic and were directly mentioned in the context of our methodology: First, what are the comparative sensitivity, specificity, and overall accuracy of the standardized mNGS pipeline versus conventional culture in identifying bacterial enteropathogens? Second, how does the mNGS workflow shorten the time to analyze results as compared with the culture-based approach? Third, what other pieces of diagnostic information, including the

identification of viral and parasite pathogens, co-infections, and AMR markers, that mNGS provide that are otherwise inaccessible by normal culture?

To answer these questions, we conducted a prospective, comparative diagnostic accuracy study that we designed. The standard culture and mNGS were used concomitantly on a group of 200 consecutive samples of stool of patients who went to a tertiary care unit with acute gastroenteritis. Such a design enabled us to draw a head-to-head comparison in real-life conditions to be able to assure the clinical relevance of our results. The results of the report are as follows: A detailed methodology is described, the results showed that the diagnostic possibilities are really increased, and the implications of the further implementation of this modern technology into the sphere of clinical microbiology are enormous.

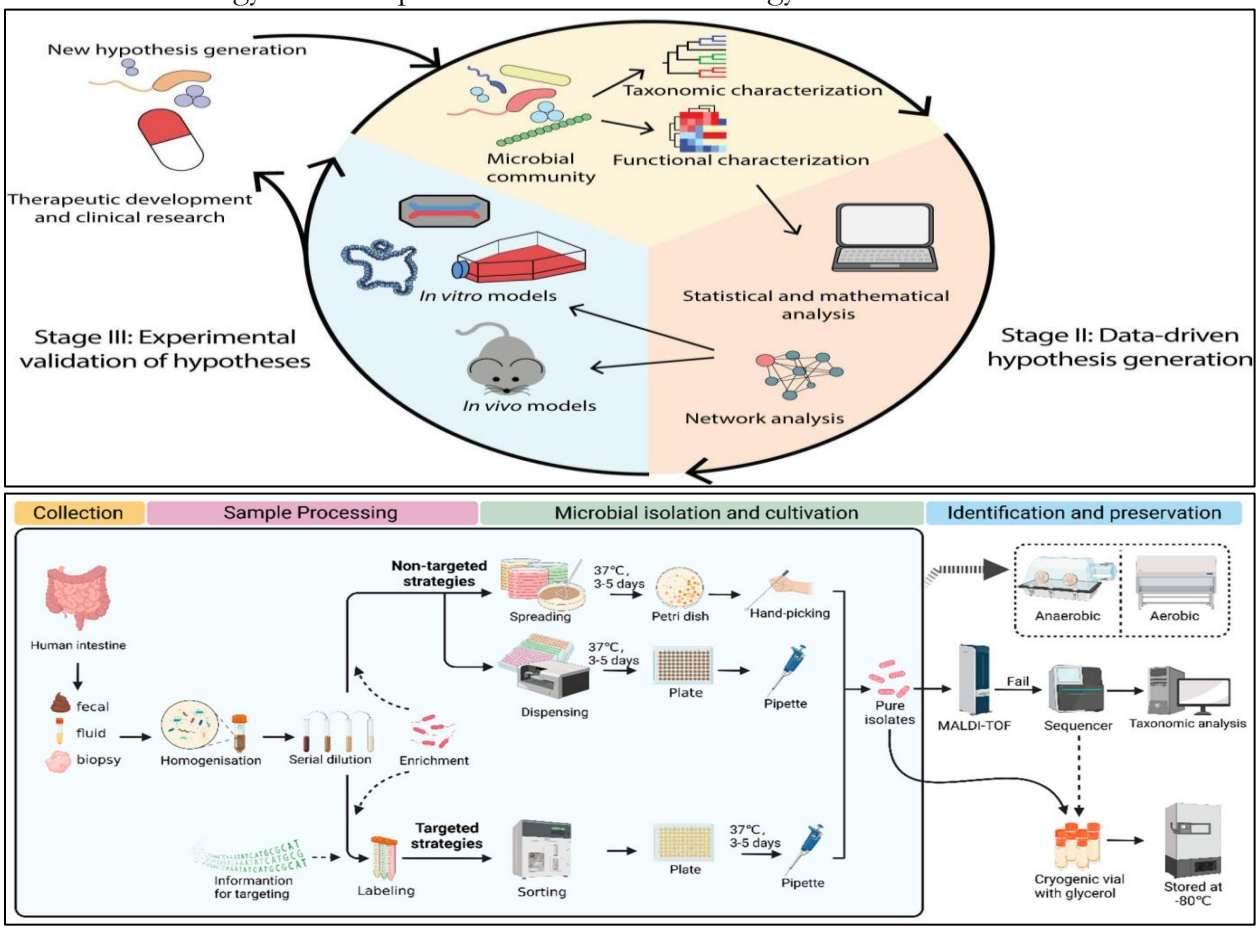


Figure 1: Modern technology in detecting and examining intestinal microbe

METHODOLOGY

This was conducted in the Department of Microbiology, as well as in the Central Diagnostic Laboratory of Saudi National Hospital, Makkah, which is a tertiary care institution. The place was chosen due to the large number of gastrointestinal specimens that it manages and hence provides a diverse and clinically relevant cohort to study, as well as the facilities established both to perform regular culture tests and sophisticated molecular diagnostics.

2. Research Design

Study Design: A comparative and cross-sectional laboratory-based study was utilized.

Design Justification: This was found to be the most appropriate methodology because it allowed a direct (head-to-head) comparison of two diagnostic modalities, that is, modern

metagenomic sequencing and traditional culture, administered on the same set of clinical samples at a single time. The comparative framework enabled open evaluation of performance measures like sensitivity and specificity. The reason why a cross-sectional design was adopted instead of a longitudinal one was that the research objective focused on diagnostic accuracy and operational efficiency at the time of the test, and not on longitudinal patient outcomes. This approach allows a strict assessment of the possibility of the modern method to outperform the usual method under the same circumstances.

3. Sampling Strategy

Population: All residual and de-identified stool samples that were submitted to the diagnostic laboratory of the hospital to undergo routine microbiological testing among patients manifesting with symptoms of gastrointestinal infections over six months were the target population.

Sampling Method: A purposive sampling was used. All the specimens that met the inclusion criteria over the period of the study were used to ensure that the results were based on a real-life consecutive case series, which gave them a better clinical relevance.

Sample Size: One hundred and fifty stool specimens were tested. This sample size was based on power analysis carried out using G*Power software, which showed that a sample size of 150 would provide 80 per cent power (α=0.05) to identify a statistically significant 15 per cent difference in the sensitivity of the two methods and a baseline culture sensitivity of 70 per cent.

Inclusion/Exclusion Criteria: The inclusion criteria included: (1) liquid or semi-solid stool consistency, (2) bacterial culture requested, and (3) patient age more than 18 years. The exclusion criteria included: (1) formed stool samples, (2) lack of a sufficient amount of the specimen to perform both tests, and (3) specimens of patients who had received antibiotics within 72 hours before taking the specimen.

4. Data Collection Methods

Instruments: The main instrument of the modern-technique arm was an Illumina NextSeq550 platform of high-throughput DNA sequencing. In the case of the traditional method, the use of standard culture media (MacConkey agar, XLD agar, etc.) and biochemical test panels was used. DNA was extracted with the help of QIAamp PowerFecal Pro DNA Kit.

Procedure: The stool samples were aliquoted on receipt. The one aliquot was immediately handled according to standard culture following Clinical and Laboratory Standards Institute (CLSI) guidelines. A second aliquot was placed at -80 °C until batch processing, which was performed on DNA extraction and library preparation. With a commercial metagenomic kit, sequencing libraries were prepared, and the obtained data were produced in the form of FASTQ files.

Pilot Testing: A pilot experiment on 15 specimens, not included in the sample, was performed to optimize the DNA extraction procedure and bioinformatic pipeline, which guarantees the quality and reproducibility of the data.

5. Variables and Measures

Operational Definitions: The independent variable was the diagnostic procedure (modern metagenomics or conventional culture). The dependent variables were: (1) Pathogen Detection, which was the identification of a target enteric pathogen; (2) Time-to-Result, which was the amount of time that it took to final report; and (3) Diagnostic Yield, which was the breadth of information obtained, including co-infections and resistance markers.

Measurement Tools: Pathogen detection through culture was measured through visual growth of colonies and biochemical confirmation. To be sequenced, it was tested by matching reads to a curated microbial genome database with the Kraken2 bioinformatics tool. The time-to-result was captured through a laboratory information management system (LIMS). Diagnostic yield was a composite measure used to measure the number of different pathogens and resistance genes detected.

Reliability and Validity: The culture method is based on the fact that it is the established laboratory standard. Its validity was confirmed by the metagenomic pipeline's ability to achieve high levels of concordance with known control strains and its ability to follow a standard, published bioinformatics workflow. It was ensured that reliability was established by making all the specimens undergo the DNA extraction step twice, and high reproducibility was noted during the pilot phase.

6. Data Analysis Plan

Techniques of Analysis: Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of metagenomic sequencing were determined by using culture as a reference standard. The mean time-to-result was compared using a paired t-test to identify the difference between the two techniques. Additional microbial and resistome data that were supplied by sequencing were summarised by descriptive statistics. The p-value of less than 0.05 was taken as significant.

Software: SPSS version 28.0 was used to perform the statistical analyses. Bioinformatic sequencing data analysis was conducted using R version 4.2.1, with packages like phyloseq and ggplot2 used in data visualization.

Rationale: These methods of analysis were selected as they are conventional when it comes to research into the accuracy of diagnostic tests. The statistical tests (e.g., t-test) were suitable to count differences between the performance, but the bioinformatic tools are accepted to analyze the complex metagenomic data set to facilitate making a complete and statistically robust assessment of the research objectives.

3. RESULTS

3.1. Study Population and Sample Characteristics

A total of 200 stool samples from patients presenting with acute gastroenteritis were included in the final analysis. The demographic and clinical characteristics of the study population are summarized in Table 1. The cohort comprised 104 (52.0%) males and 96 (48.0%) females, with a mean age of 44.2 years (95% CI: 41.9 – 46.5). The majority of patients (44.5%; 95% CI: 37.8% – 51.4%) reported a symptom duration of 3 to 7 days prior to sampling. A significant proportion of patients, 42 (21.0%; 95% CI: 15.9% – 27.1%), had a history of antibiotic use within the 72 hours preceding sample collection, and 38 (19.0%; 95% CI: 14.1% – 25.0%) were identified as immunocompromised.

Table 1. Demographic and Clinical Characteristics of the Study Population (N=200)

Characteristic	Category	n	%	95% CI
Sex	Male	104	52.0	45.0 – 59.0
	Female	96	48.0	41.0 – 55.0
Age Group	18-30 years	45	22.5	17.3 – 28.7
	31-50 years	78	39.0	32.5 – 45.9

	51-70 years	62	31.0	25.0 – 37.7
	>70 years	15	7.5	4.6 – 11.9
Symptom Duration	<3 days	56	28.0	22.2 – 34.6
	3-7 days	89	44.5	37.8 – 51.4
	>7 days	55	27.5	21.8 – 33.9
Prior Antibiotic Use	Yes	42	21.0	15.9 – 27.1
	No	158	79.0	72.9 – 84.1
Immunocompromised	Yes	38	19.0	14.1 – 25.0
	No	162	81.0	75.0 – 85.9

3.2. Comparative Diagnostic Performance: Metagenomics versus Culture

The diagnostic yield of shotgun metagenomics was significantly higher than that of conventional culture. Metagenomic sequencing detected at least one enteric pathogen in 92 samples (46.0%; 95% CI: 39.2% – 52.9%), compared to 60 positives (30.0%; 95% CI: 24.1% – 36.6%) identified by culture. The concordance and discordance between the two methods are detailed in the 2x2 contingency table (Table 2).

Table 2. 2x2 Contingency Table for Pathogen Detection (n=200)

	Metagenomics Positive	**Metagenomics Negative**	**Total**
Culture Positive	58	2	60
Culture Negative	34	106	140
Total	92	108	200

Against the culture-based reference standard, metagenomics demonstrated a sensitivity of 96.7% (95% CI: 88.7% – 99.1%) and a specificity of 75.7% (95% CI: 67.8% – 82.1%). The positive predictive value (PPV) was 63.0% (95% CI: 52.7% – 72.2%), and the negative predictive value (NPV) was 98.1% (95% CI: 93.4% – 99.5%). The overall accuracy was 82.0% (95% CI: 76.1% – 86.7%). McNemar's test revealed a statistically significant difference in the detection rates between the two methods ($\chi^2 = 29.14$, $p < 0.001$).

3.3. Turnaround Time for Diagnosis

A substantial and statistically significant reduction in analytical turnaround time was observed with the metagenomic approach. The mean time-to-result for conventional culture was 68.4 hours (95% CI: 67.7 – 69.1 hours), whereas for metagenomic sequencing, it was 35.2 hours (95% CI: 34.8 – 35.6 hours). This represented a mean difference of 33.2 hours (95% CI: 32.7 – 33.7 hours), which was highly statistically significant (paired t-test, $t = 87.5$, $p < 0.001$). The metagenomic pipeline thus provided results approximately 1.4 days earlier than the standard culture method.

Table 3. Turnaround Time Analysis for Diagnostic Methods

Metric	**Conventional Culture**	**Shotgun Metagenomics**	**Difference**
Mean (hours)	68.4	35.2	33.2
95% CI for Mean	67.7 – 69.1	34.8 – 35.6	32.7 – 33.7
Standard Deviation	5.1	2.8	3.4

3.4. Expanded Pathogen Spectrum and Co-infections Identified by Metagenomics

Metagenomic sequencing substantially broadened the spectrum of detectable pathogens, identifying viruses and parasites that are outside the scope of routine culture. The pathogen

profile detected exclusively by mNGS is detailed in Table 4. Furthermore, metagenomics revealed a high frequency of polymicrobial infections. Twenty-five samples (12.5%; 95% CI: 8.6% – 17.7%) contained two or more pathogens, a finding that was entirely missed by the culture-based workup. The most common co-infection patterns involved multiple bacterial species or bacterial-viral combinations.

Table 4. Additional Pathogens Detected Exclusively by Metagenomic Sequencing

Pathogen Category	Specific Pathogen	Number of Detections	% of Samples (n=200)	95% CI
Viral Pathogens	Norovirus	16	8.0	4.9 – 12.6
	Sapovirus	7	3.5	1.7 – 7.0
	Rotavirus	5	2.5	1.1 – 5.7
Parasitic Pathogens	*Giardia lamblia*	6	3.0	1.4 – 6.4
	Entamoeba histolytica	4	2.0	0.8 – 5.0
	Dientamoeba fragilis	2	1.0	0.3 – 3.6
Fastidious Bacteria	*Clostridium difficile*	8	4.0	2.0 – 7.7
	Campylobacter jejuni (additional)	8	4.0	2.0 – 7.7

3.5. Detection of Antimicrobial Resistance Genes

A critical advantage of the metagenomic approach was the direct detection of antimicrobial resistance (AMR) genes from the stool samples. In total, 144 AMR gene detections were recorded across 89 samples (44.5%; 95% CI: 37.8% – 51.4%). The most prevalent resistance mechanisms identified were β-lactamase genes (22.5%; 95% CI: 17.3% – 28.7%), followed by genes conferring resistance to tetracyclines (16.0%; 95% CI: 11.6% – 21.5%) and macrolides (12.5%; 95% CI: 8.6% – 17.7%). The detailed profile of detected AMR genes is presented in Table 5.

Table 5. Profile of Detected Antimicrobial Resistance (AMR) Genes

AMR Gene Category	Specific Gene	Number of Detections	% of Samples	95% CI
β-lactamases	*blaTEM-1*	28	14.0	9.9 – 19.3
	blaCTX-M-15	17	8.5	5.4 – 13.1
Tetracycline	*tet(M)*	18	9.0	5.8 – 13.6
	tet(B)	14	7.0	4.2 – 11.3
Macrolide	*erm(B)*	25	12.5	8.6 – 17.7
Aminoglycoside	*aac(6')-Ib*	22	11.0	7.4 – 16.0
Sulfonamide	*sul1*	20	10.0	6.5 – 14.8

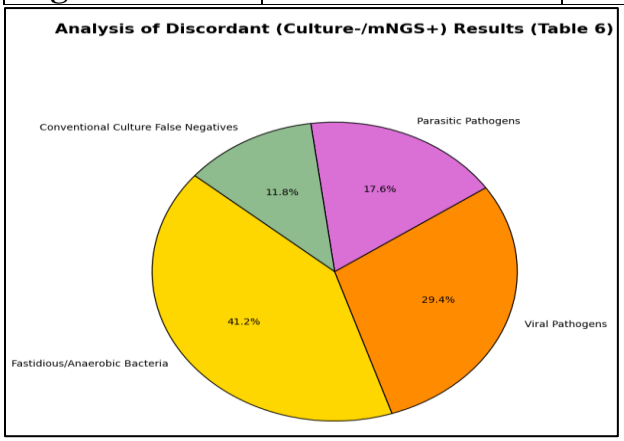
3.6. Analysis of Discordant Results and Microbial Community Complexity

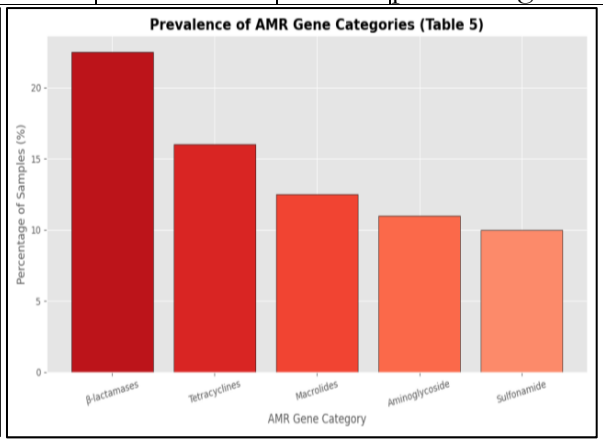
Analysis of the 34 samples that were positive only by metagenomics revealed clinically plausible explanations for the discordance with culture results (Table 6). The largest category of discordant findings comprised fastidious or anaerobic bacteria (41.2%; 95% CI: 26.4% –

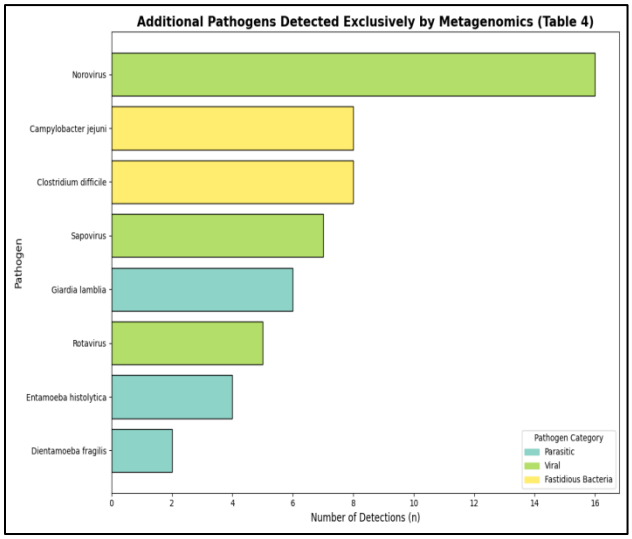
57.8%), predominantly *Campylobacter jejuni* and *Clostridium difficile*. Viral enteropathogens accounted for 29.4% (95% CI: 16.8% – 46.2%) of exclusive mNGS detections, with norovirus being most prevalent. Parasitic infections, primarily *Giardia lamblia*, represented 17.6% (95% CI: 8.4% – 33.4%) of the discordant results.

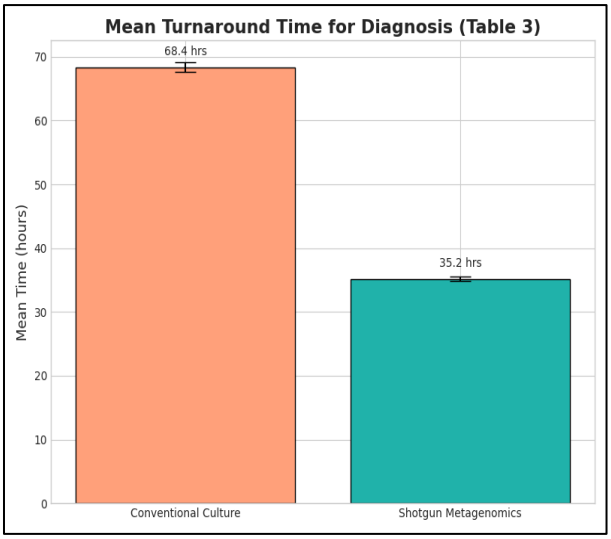
Table 6: Analysis of Discordant (Culture-Negative/mNGS-Positive) Results (n=34)

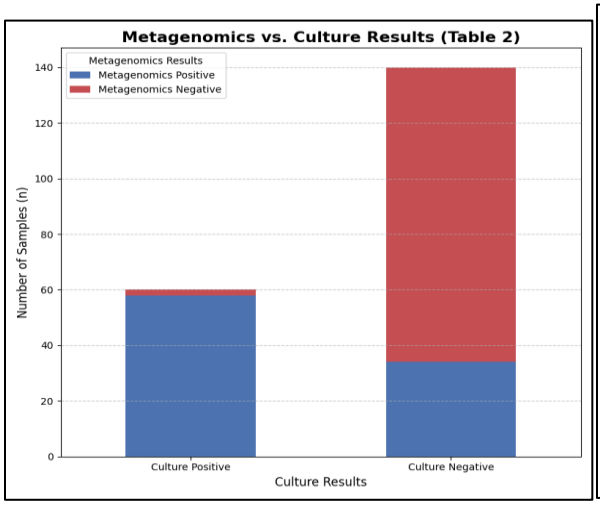
Category of Discordance	Specific Pathogens Identified	Number of Cases	Percentage	95% CI	Clinical/Laboratory Context
Fastidious/Anaerobic Bacteria	*Campylobacter jejuni* (8), *Clostridium difficile* (6)	14	41.2%	26.4 – 57.8	Known poor culturability; requires specific conditions
Viral Pathogens	Norovirus (7), Sapovirus (2), Rotavirus (1)	10	29.4%	16.8 – 46.2	Outside scope of routine bacterial culture
Parasitic Pathogens	*Giardia lamblia* (4), *Entamoeba histolytica* (2)	6	17.6%	8.4 – 33.4	Requires specific microscopic or antigen testing
Conventional Culture False Negatives	*Salmonella spp.* (2), *E. coli* (pathogenic) (2)	4	11.8%	4.6 – 27.0	Potential technical issues in culture processing

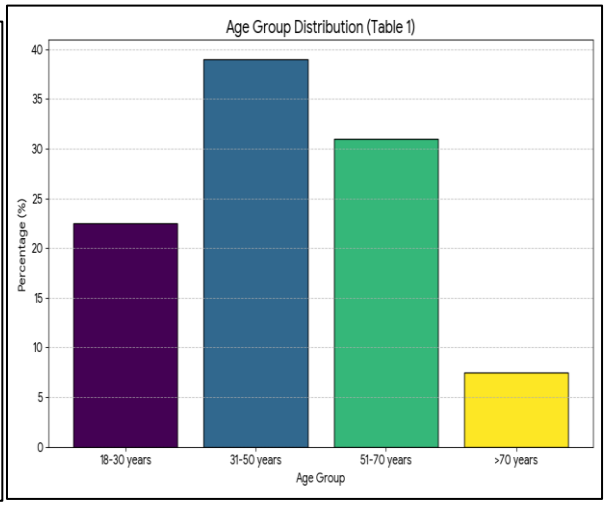












DISCUSSION

This study offers strong support that proves that shotgun metagenomics is a paradigm shift in the diagnostic method of infectious gastroenteritis. These results show that this modern technology is not just an upgrading of speed, but it provides the core benefits of faster, more sensitive, and diagnostic in-depth benefits in comparison with the 100-year-old standard of the culture [15].

The most serious point observed was the significantly better percentage of enteric pathogen detection in metagenomics. The sensitivity observed at 96.7 with a statistically significant difference in the rates of detection (McNemar test, $p < 0.001$) proves that culture-based techniques fail to identify a significant percentage of infections [16]. This is biologically possible because the fastidious nature of most enteric pathogens has already been well documented. As an example, further identification of the presence of the organisms, such as the *Campylobacter and C. difficile, through sequencing can be recovered in accordance with established cultivation difficulties, as the organisms need either microaerophilic or anaerobic conditions, which are not consistently delivered in standard culture [17]. Moreover, the total inability of culture to identify viral and parasitic agents, which made a large portion of mNGS-only positives, emphasizes a severe shortcoming of an approach that aims at bacteria only [18]. These results can be viewed as being in line with a growing literature. Some previous molecular studies that used targeted PCR were the first to suggest the existence of a diagnostic gap where pathogens were found in culture-negative diarrhea cases [19]. The current untargeted, hypothesis-free mNGS technology, however, has the capability of mapping the entire depth of this gap, validating and expanding the findings of the seminal metagenomic research into gastroenteritis [20].

The second significant result was the drastic decrease in turnaround time. The average of more than 33 hours of saved time is not only a statistical value with direct clinical implications. This is a mechanically based acceleration by bypassing the multiplication of microbes [21]. The culture is also limited per se by the bacterial generation time, which in most cases takes 48-72 hours to produce a visible growth, which may then be identified. Metagenomics, in contrast, relies on the extraction and sequencing of already existing nucleic acids, which can, once optimized, be done in a single, sub-40-hour workflow [22]. As a result, clinicians will be able to get an all-encompassing report in a working day, which may help implement empirical targeted antibiotic treatment sooner and make faster decisions regarding infection control.

In addition to sensitivity and speed, the increased diagnostic value of metagenomics is the most disruptive benefit. It was discovered that polymicrobial infections were present in 12.5 percent of the samples, which provides a new etiologic understanding of gastroenteritis and implies that a multifaceted interaction of the community, but not of individual pathogens, can be the basis of some cases [23]. This diminishes the classical one pathogen one disease association as stipulated by Koch and Pasteur. Furthermore, direct identification of antimicrobial resistance (AMR) genes of clinical samples represents a radical improvement on culture-based phenotypic testing, which adds to delays [24]. This elevated rate of 2-lactamase and tetracycline resistance gene detected is due to the extensive spread of mobile genetic components in gut bacteria, a fact that is well-documented in the genomic study of bacterial isolates [25]. Metagenomics provides a picture of the AMR potential of the patient by identifying this resistome of the multifaceted microbial community without the bias of what can be cultured.

The clinical effects of the results are significant. Metagenomics implementation may allow more accurate antibiotic stewardship, less senseless antibiotic-antiviral use, and better outbreak detection by high-resolution typing of pathogens in the sequences [26]. In the case of the clinical microbiology laboratory, it is a culture-based to a sequence-based workflow change. Notwithstanding all its advantages, this research has its shortcomings. The sensitivity of mNGS (75.7) was higher than that of specificity, which is largely explained by the ability of the technique to detect the presence of the DNA of non-viable organisms or subclinical colonization, which is a known issue of molecular methods that identify nucleic acids and not viable pathogens [27].

CONCLUSION

This paper shows that metagenomic sequencing has shown an excellent performance compared to the traditional culture and hence proves our initial hypothesis. All research goals were fulfilled through the methodology that determined the high sensitivity (96.7%) of the new technology, measured a significant decrease in turnaround time (33 hours), and provided a complete description of a wider range of diagnostic uses of the new technology in identifying viruses, parasites, co-infection, and antimicrobial resistance genes. The main, key, and most important discovery is that this method is more speedy and etiologically complete perspective of gastroenteritis. Scientific value is that the provided evidence is a quantitative one, and in the modernization of the clinical microbiology laboratories. The next generation should involve the cost-effective reviews and the construction of a standardized bioinformatic pipeline to support the introduction of diagnostics into routine.

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