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Review article

Microbiological methodologies: Comparative evaluation of microbial community and enhanced antibiotic susceptibility testing ☆,☆☆

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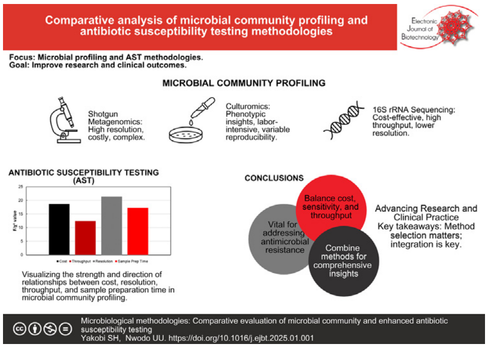
GRAPHICAL ABSTRACT



Comparative analysis of microbial community profiling and antibiotic susceptibility testing methodologies

Focus: Microbial profiling and AST methodologies.
Goal: Improve research and clinical outcomes.

MICROBIAL COMMUNITY PROFILING

Shotgun Metagenomics: High resolution, costly, complex.

Culturomics: Phenotypic insights, labor-intensive, variable reproducibility.

16S rRNA Sequencing: Cost-effective, high throughput, lower resolution.

ANTIBIOTIC SUSCEPTIBILITY TESTING (AST)

Visualizing the strength and direction of relationships between cost, resolution, throughput, and sample preparation time in microbial community profiling.

CONCLUSIONS

Balance cost, sensitivity, and throughput

Vital for addressing antimicrobial resistance

Combine methods for comprehensive insights

Advancing Research and Clinical Practice
Key takeaways: Method selection matters; integration is key.

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ABSTRACT

Background: This study provides a comparative analysis of microbial community profiling and antibiotic susceptibility testing (AST) methodologies.

Microbial community profiling: Methods such as Shotgun Metagenomics and 16S rRNA sequencing were evaluated based on criteria including resolution, throughput, cost, and reproducibility. Shotgun Metagenomics was found to offer the highest resolution and detailed insights into microbial diversity, though at a higher cost and complexity. In contrast, 16S rRNA Sequencing provided a more cost-effective and high-throughput alternative, suitable for large-scale studies despite lower taxonomic resolution. Culturomics, while offering unique phenotypic data, showed variability in reproducibility and required more labor-intensive processes.

Antibiotic susceptibility testing (AST): Traditional methods such as disk diffusion and broth microdilution were compared to emerging molecular and automated AST technologies. Traditional methods were noted for their precision in determining minimum inhibitory concentrations (MICs), crucial for guiding effective antimicrobial therapy. However, the emerging methods provided faster turnaround times and higher throughput, which are increasingly important in clinical settings focused on antimicrobial stewardship.

Conclusions: The study underscores the importance of selecting appropriate methodologies based on specific research or clinical needs, balancing factors such as cost, sensitivity, and throughput. The integration of multiple methodologies is recommended to overcome the limitations of individual techniques,

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☆ Audio abstract available in Supplementary material.

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providing a more comprehensive understanding of microbial ecosystems and resistance profiles. These findings are crucial for enhancing both research and clinical practices, particularly in the context of the global challenge posed by antimicrobial resistance.

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Contents

1. Introduction

The field of microbiology has seen a paradigm shift over the past few decades, driven by the pressing need for more accurate, efficient, and comprehensive tools for detecting, profiling, and combating pathogens [1,2]. Traditional microbiological techniques, while foundational, often struggle with limitations such as prolonged culture times, low sensitivity, and the inability to detect non-culturable organisms [3]. These constraints have significant implications, especially in the context of emerging infectious diseases and the growing global threat of antimicrobial resistance (AMR) [4,5]. To address these challenges, the development and integration of innovative methodologies such as molecular diagnostics, next-generation sequencing (NGS), and high-throughput phenotypic assays have become critical. The advent of next-generation sequencing (NGS) has further expanded the capabilities of molecular diagnostics [6]. NGS enables whole-genome sequencing of pathogens, providing comprehensive data on genetic variations, resistance mechanisms, and phylogenetic relationships [7,8]. This technology has been instrumental in outbreak investigations, tracking the spread of infectious agents, and understanding the evolution of AMR [9]. Metagenomics, a subset of NGS, allows for the simultaneous analysis of entire microbial communities directly from environmental or clinical samples, bypassing the need for culture and providing insights into the microbiome's role in health and disease [10]. The characterization of microbial communities has become increasingly important in understanding the complexity of infections, particularly in the context of the human microbiome [11]. Techniques such as 16S rRNA sequencing have been widely adopted for identifying and quantifying bacterial populations within a sample, providing valuable insights into the diversity and dynamics of microbial communities [12]. However, 16S rRNA sequencing is limited to bacterial identification and does not capture the full diversity of viruses, fungi, and other microorganisms present in a sample. Shotgun metagenomics, which sequences all genetic material within a sample, offers a more comprehensive view of microbial communities, including the detection of viruses, bacteria, fungi, and archaea [13,14,15]. This approach has been particularly useful in studying polymicrobial infections and the impact of the microbiome on health and disease [13]. Microarray-based technologies, which allow for the simultaneous detection of multiple microbial species and resistance genes, have also been developed, offering high-throughput and multiplexed capabilities [16]. Antibiotic susceptibility testing (AST) remains a critical component of microbiological diagnostics, guiding the selection of appropriate antimicrobial therapies. Traditional AST methods, such as disk diffusion (Kirby-Bauer) and broth microdilution, have been the gold standard for determining the susceptibility of bacteria to antibiotics. These methods, while reliable, are labor-intensive and time-consuming, often taking 24–48 h to yield results (Clinical and Laboratory Standards Institute 2020 standards) [17,18]. To address the need for faster and more accurate AST, automated systems and genotypic resistance testing have been developed. Automated AST systems, such as the VITEK 2 and BD Phoenix, provide rapid and automated minimum inhibitory concentration (MIC) determinations, improving throughput and reducing labor [19]. Genotypic resistance testing, which detects specific resistance genes through molecular methods, offers a rapid alternative to phenotypic methods and has been particularly useful in detecting multidrug-resistant organisms [20,21]. High-throughput screening techniques, which enable the simultaneous testing of multiple antibiotics against a wide range of pathogens, are also emerging as powerful tools in the fight against AMR. These methods facilitate the discovery of novel resistance patterns and the identification of potential therapeutic combinations, providing a new frontier in the battle against resistant infections [22]. As microbiology continues to evolve, the integration of traditional and modern methodologies is essential to improving diagnostic accuracy, patient outcomes, and public health. The convergence of culture-based methods, molecular diagnostics, and high-throughput technologies offers the potential for more comprehensive and personalized approaches to infectious disease management. Moreover, the ongoing development of novel techniques, such as CRISPR-based diagnostics and single-cell genomics, promises to further enhance our ability to detect, profile, and combat pathogens [23].

In this article, we will provide a comparative analysis of traditional and emerging microbiological techniques, focusing on their applications in microbial community profiling and antibiotic susceptibility testing. We will examine the strengths and limitations of each approach, highlighting recent innovations and discussing their implications for clinical practice and research.

2. Microbial community profiling

Microbial community profiling is an essential tool in understanding the composition, diversity, and dynamics of microbial populations in various environments, including human microbiomes, soil, water, and industrial settings [24]. Unlike traditional pathogen detection, which focuses on identifying specific pathogens, microbial community profiling provides a comprehensive overview of all microorganisms present in a sample, offering insights into their interactions, functions, and potential roles in health and disease.

2.1. 16S rRNA gene sequencing: A molecular census of bacteria

16S ribosomal RNA (rRNA) gene sequencing is one of the most widely used methods for profiling bacterial communities. The 16S rRNA gene, present in all bacteria and archaea, contains conserved and hypervariable regions that allow for the identification and classification of bacteria at different taxonomic levels [25]. The process begins with the extraction of total DNA from a sample, followed by the amplification of the 16S rRNA gene using PCR. The amplified gene regions, typically covering hypervariable regions such as V3-V 4 or V4-V 5, are then sequenced using high-throughput sequencing platforms like Illumina MiSeq [26]. The resulting sequences are compared against reference databases (SILVA, Greengenes, or RDP) to assign taxonomic identities to the detected bacteria [14,26]. 16S rRNA sequencing is highly sensitive and allows for the detection of both abundant and rare bacterial taxa. It is also culture-independent, enabling the analysis of unculturable or difficult-to-culture bacteria, which are often missed by traditional methods [27]. Despite its utility, 16S rRNA sequencing has limitations. The resolution is generally limited to the genus or species level, and closely related species may not be distinguishable. Additionally, it provides limited information on the functional capabilities of the microbial community, as it focuses solely on the 16S rRNA gene [28].

2.2. Metagenomics: Comprehensive analysis of microbial genomes

Metagenomics involves the sequencing of all genetic material present in a sample, providing a more comprehensive view of the microbial community compared to 16S rRNA sequencing. This approach captures not only bacterial genomes but also those of viruses, fungi, and other microorganisms, offering insights into the entire microbial ecosystem [10]. In metagenomics, total DNA is extracted from the sample and subjected to high-throughput sequencing without prior amplification of specific genes. The resulting sequences are assembled into contigs and analyzed using bioinformatics tools to identify microbial species, functional genes, and metabolic pathways. Metagenomics can be shotgun (random sequencing of all DNA) or targeted (sequencing specific functional genes or regions) [10,15]. Metagenomics provides a holistic view of the microbial community, allowing for the identification of not only the organisms present but also their functional potential. This approach is particularly valuable in studying complex ecosystems, identifying novel microbes, and understanding microbial interactions and metabolic networks [14,29]. Metagenomics is more resource-intensive than 16S rRNA sequencing, requiring significant computational power and expertise in bioinformatics. The large volume of data generated can be challenging to analyze and interpret, and there is a risk of missing low-abundance species due to the dominance of more abundant organisms in the sequencing data.

2.3. Metatranscriptomics: Profiling active microbial communities

While metagenomics provides information on the potential functions of a microbial community, metatranscriptomics focuses on the active functions by analyzing the community's RNA, particularly messenger RNA (mRNA) [30]. This approach reveals which genes are being expressed at the time of sampling, offering insights into the active metabolic pathways and interactions within the community. Metatranscriptomics begins with the extraction of total RNA from the sample, followed by the removal of ribosomal RNA (rRNA) to enrich for mRNA. The mRNA is then converted into complementary DNA (cDNA) and sequenced using high-throughput sequencing platforms. The resulting sequences are mapped to reference genomes or metagenomic assemblies to identify expressed genes and pathways [31,32]. Metatranscriptomics provides a real-time snapshot of microbial activity, revealing the functional state of the community and its response to environmental changes. It is particularly useful in studying microbial responses to stress, infection, or treatment, and in exploring the dynamics of microbial interactions [33]. The main challenges of metatranscriptomics include the instability of RNA, which can degrade rapidly, and the technical difficulties in extracting high-quality RNA from complex samples. Additionally, the presence of abundant rRNA can complicate data analysis, despite efforts to remove it. Metatranscriptomics also requires advanced bioinformatics tools for data processing and interpretation [31,32].

2.4. Metaproteomics: Understanding microbial function through proteins

Metaproteomics is the study of the entire protein complement expressed by a microbial community. It provides direct evidence of microbial functions and interactions by identifying and quantifying proteins involved in metabolic pathways, cellular processes, and microbial interactions [33]. Proteins are extracted from the microbial community, digested into peptides, and analyzed using mass spectrometry (MS). The resulting peptide spectra are compared against protein databases or metagenomic sequences to identify and quantify the proteins present. Metaproteomics can provide insights into the functional activity of the community and its response to environmental or physiological conditions [34,35]. Metaproteomics offers a direct measure of microbial activity by focusing on the proteins responsible for various functions within the community. It can reveal post-translational modifications, protein–protein interactions, and the presence of enzymes and other functional proteins that may not be detected at the genetic or transcriptomic level [34]. Metaproteomics is technically complex and requires sophisticated instrumentation and expertise in mass spectrometry and proteomics. The interpretation of metaproteomic data can be challenging due to the complexity of microbial communities and the vast diversity of proteins [36,37]. Additionally, the dynamic range of protein expression can make it difficult to detect low-abundance proteins [36].

2.5. Metabolomics: Profiling microbial metabolites

Metabolomics involves the comprehensive analysis of metabolites produced by a microbial community, providing insights into its metabolic activities and interactions with the environment [30]. Metabolites are the end products of cellular processes, and their analysis can reveal the functional state of the community and its influence on the surrounding environment. Metabolites are extracted from the sample and analyzed using techniques such as gas chromatography-mass spectrometry (GC–MS) or liquid chromatography-mass spectrometry (LC-MS). The resulting metabolite profiles are compared against reference libraries to identify and quantify the metabolites present [38,39]. Metabolomics can provide insights into microbial metabolism, nutrient cycling, and the production of bioactive compounds [40]. Metabolomics offers a direct measure of the metabolic output of a microbial community, providing insights into its functional state and interactions with the environment. It is particularly useful in studying microbial contributions to health and disease, bioremediation, and the production of industrially relevant compounds [41]. The interpretation of metabolomic data can be challenging due to the complexity of metabolic networks and the influence of environmental factors [42]. Metabolomics also requires advanced instrumentation and expertise in analytical chemistry, and the detection of metabolites can be influenced by their stability and extraction efficiency [43].

2.6. Integration of multi-omics approaches

While each of the microbial community profiling techniques described above provides valuable insights into different aspects of microbial communities, the integration of these approaches, often referred to as multi-omics, offers a more comprehensive understanding of microbial ecosystems [44]. Multi-omics approaches combine data from metagenomics, metatranscrip-

Table 1
A Comparative analysis key characteristics of microbial community profiling techniques.

Parameter	16S rRNA Gene Sequencing [13,28]	Metagenomics [15,16]	Metatranscriptomics [31,32]	Metaproteomics [34,34]	Metabolomics [41,42]
Target Biomolecule	16S rRNA gene (bacteria and archaea)	Entire genomic DNA	mRNA (expressed genes)	Proteins	Metabolites
Resolution Level	Genus/Species	Species/Strain level	Gene expression level	Protein function and abundance	Metabolic pathways and profiles
Specificity	Moderate	Very High	High	High	Moderate to High
Sensitivity	High	Very High	High	Moderate to High	Moderate
Data Output	Taxonomic profiles	Taxonomic and functional profiles	Gene expression profiles	Protein abundance and function	Metabolite abundance and profiles
Required Sequencing Depth	Moderate	High	High	Very High	High
Turnaround Time	Moderate	Long	Long	Long	Moderate
Cost	Moderate	High	High	Very High	High
Computational Demand	Moderate	High	Very High	Very High	High
Bioinformatics Complexity	Moderate	High	Very High	Very High	High
Sample Preparation	Simple to Moderate	Complex	Complex	Very Complex	Complex
Advantages	Cost-effective, detects unculturable bacteria	Comprehensive community analysis, novel species detection	Real-time activity insights	Direct measure of function, protein interactions	Direct measure of metabolic output
Limitations	Limited functional insights, genus-level resolution	Expensive, complex data analysis	RNA instability, high technical complexity	Expensive, challenging data interpretation	Metabolite instability, complex analysis

*Very High: >90%; High: 70–90%; Moderate to High: 60–70%; Moderate: 50–60%.

tomics, metaproteomics, and metabolomics to provide a holistic view of microbial communities, from genetic potential to actual function and metabolic output [30,44]. Integrating multi-omics data can reveal complex interactions within microbial communities, such as the relationship between genetic potential (metagenomics), gene expression (metatranscriptomics), protein function (metaproteomics), and metabolic activity (metabolomics). This approach is particularly powerful in studying the microbiome, environmental microbiology, and host-microbe interactions [45]. The main challenges of multi-omics approaches include the need for large-scale data integration and interpretation, as well as the high cost and complexity of generating and analyzing multi-omics data. Additionally, the integration of data from different omics platforms requires sophisticated bioinformatics tools and expertise [31]. Microbial community profiling has evolved significantly with the advent of high-throughput sequencing and multi-omics approaches. Each profiling technique offers unique insights into microbial communities, from their composition and diversity to their functional capabilities and metabolic activities [44,46]. The comparison detailed in Table 1 underscores the importance of selecting the appropriate microbial community profiling technique based on the research objectives, available resources, and desired level of detail. 16S rRNA sequencing is well-suited for broad taxonomic studies and cost-effective analyses, while metagenomics provides the most comprehensive view of microbial communities but at a higher cost and complexity. Metatranscriptomics and metaproteomics are invaluable for studying microbial activity and functional interactions, though they demand significant resources and technical expertise. Metabolomics offers critical insights into microbial metabolism but may be less detailed in terms of taxonomic resolution. These findings highlight that no single method is universally superior, rather, the choice of technique should be tailored to the specific research question, balancing factors such as specificity, sensitivity, cost, and complexity. Integrating multiple approaches (multi-omics) can provide a more holistic understanding of microbial communities, but it requires careful consideration of the trade-offs involved.

3. Antibiotic susceptibility testing (AST)

Antibiotic Susceptibility Testing (AST) is a critical process in microbiology that determines the sensitivity or resistance of bacterial strains to specific antibiotics. This testing is essential for guiding appropriate antibiotic therapy, tracking the emergence of resistance, and contributing to public health surveillance. AST methods have evolved significantly over the years, with advancements in both traditional and molecular techniques that enhance the precision, speed, and reliability of results [17,18].

3.1. Disc diffusion method (Kirby-Bauer Test)

The disc diffusion method, also known as the Kirby-Bauer test, is one of the most widely used techniques for AST. It involves placing antibiotic-impregnated paper discs on an agar plate inoculated with the bacterial isolate in question. The antibiotic diffuses into the agar, creating a gradient. As bacteria grow, they are inhibited by the antibiotic, forming a clear zone around the disc where bacterial growth is suppressed, known as the zone of inhibition. A standardized bacterial suspension is spread evenly on the surface of a Mueller-Hinton agar plate. Paper discs impregnated with specific concentrations of antibiotics are then placed on the agar. After incubation, the zones of inhibition are measured, and the bacterial strain is classified as susceptible, intermediate, or resistant based on established clinical breakpoints (CLSI, 2018). The Kirby-Bauer test is simple, cost-effective, and does not require sophisticated equipment. It is suitable for a wide range of bacteria and provides clear, interpretable results that are easily comparable across laboratories. The method is semi-quantitative and may be less accurate for determining minimum inhibitory concentrations (MICs). It is also influenced by factors such as agar composition, incubation conditions, and disc potency, which can affect reproducibility [17,18,43].

3.2. Broth microdilution method

The broth microdilution method is a quantitative AST technique that determines the MIC, the lowest concentration of an antibiotic that inhibits visible bacterial growth. This method is considered the gold standard for AST and is widely used in clinical laboratories [17]. Bacterial suspensions are added to wells in a microtiter plate containing serial dilutions of the antibiotic. After incubation, bacterial growth is assessed visually or using automated systems. The MIC is defined as the lowest concentration of antibiotic that prevents visible growth [47]. The broth microdilution method provides precise and reproducible MIC values, which are essential for guiding therapy and monitoring resistance trends. It can be adapted for high throughput testing and automated analysis. The method is more labor-intensive and time-consuming than disc diffusion, requiring careful preparation and handling of microdilution plates. It also demands more expensive reagents and equipment.

3.3. E-test (Epsilometer test)

The E-test combines the principles of both disc diffusion and broth microdilution. It uses a plastic strip impregnated with a gradient of antibiotic concentrations. When placed on an inoculated agar plate, the antibiotic diffuses into the medium, creating an ellipse-shaped zone of inhibition. The MIC is read directly from the strip where the zone intersects the scale. A standardized bacterial inoculum is spread on an agar plate, and the E-test strip is placed on the surface. After incubation, the MIC is read at the point where the elliptical inhibition zone meets the strip's scale [9,48]. The E-test is user-friendly, providing accurate and quantitative MIC results with minimal equipment. It is particularly useful for testing fastidious organisms and antibiotics where disc diffusion may be less reliable. E-tests are more expensive than traditional disc diffusion, and the gradient strips are single-use, contributing to higher costs per test. Interpretation can be subjective, especially with diffuse or trailing zones of inhibition [18].

3.4. Automated AST systems

Automated AST systems, such as the VITEK 2, Phoenix, and MicroScan, offer rapid and standardized testing by integrating bacterial identification with susceptibility testing. These systems use either broth microdilution or kinetic measurements to assess bacterial growth in the presence of antibiotics [49,50]. Bacterial suspensions are loaded into the system's proprietary cards or panels, which contain wells with different concentrations of antibiotics. The system monitors growth automatically and provides MIC values and susceptibility profiles based on pre-programmed algorithms [51]. Automated systems offer rapid turnaround times, high throughput, and consistent, standardized results. They reduce the manual workload and potential for human error, making them ideal for high-volume clinical laboratories. The cost of automated systems and their consumables is high, making them less accessible to smaller or resource-limited laboratories. Additionally, these systems may have limitations in testing certain fastidious organisms or novel antibiotics [52].

3.5. Genotypic methods for AST

Genotypic methods, such as PCR and next-generation sequencing (NGS), detect the presence of resistance genes directly from bacterial DNA, offering rapid results without the need for culture. These methods are particularly valuable for detecting resistance mechanisms, such as beta-lactamase production or mutations in target genes [7]. DNA is extracted from the bacterial isolate, and specific resistance genes are amplified using PCR or sequenced using NGS. The presence of resistance genes is then correlated with phenotypic resistance profiles [53]. Genotypic methods provide rapid results, often within hours, and can detect resistance mechanisms that may not be evident through phenotypic testing. They are particularly useful in outbreak situations and for multidrug-resistant organisms. These methods do not provide MIC values and may not detect all resistance mechanisms, especially those not associated with known genes. The cost and complexity of NGS, in particular, can be prohibitive for routine use [8,53].

3.6. Emerging and advanced AST methods

Recent advancements in AST have focused on developing rapid, point-of-care testing methods and integrating machine learning for predictive susceptibility testing. Microfluidics-based AST and nanoparticle-enhanced detection are promising areas of research that aim to reduce testing times and improve sensitivity. Machine learning algorithms are being developed to predict resistance based on phenotypic data and to integrate genotypic and phenotypic testing for more comprehensive profiles [54,55]. Antibiotic Susceptibility Testing remains a cornerstone of clinical microbiology and antimicrobial stewardship. The selection of an AST method is influenced by several factors, including the type of microorganism, the clinical context, available resources, and the desired level of detail in the results [17]. While traditional methods like disc diffusion and broth microdilution remain widely used, the advent of automated and genotypic methods has revolutionized AST, offering faster, more accurate, and comprehensive results. As antibiotic resistance continues to evolve, the development and refinement of AST methods will be crucial in ensuring effective treatment and combating the spread of resistant pathogens [18].

Table 2 provides a comprehensive comparison of various AST methods, highlighting their unique advantages, limitations, and clinical applications. Understanding these differences is crucial for selecting the appropriate method based on the clinical context, laboratory resources, and the specific bacterial pathogen involved. Despite Disc Diffusion (Kirby-Bauer) being an older method, it remains widely used due to its reliability and cost-effectiveness. It is suitable for routine testing, particularly in resource-limited settings. The Broth Microdilution is the gold standard for determining MIC values, which are essential for guiding precise antibiotic therapy. Its reproducibility and accuracy make it indispensable in clinical laboratories, although it is more labor-intensive. The E-test combines the precision of MIC determination with the reliability of diffusion-based methods, making it particularly well-suited for testing fastidious organisms. However, the higher cost may limit its routine use. The Automated AST Systems offer rapid and standardized results, making them ideal for high-volume laboratories. They are particularly valuable in clinical settings where quick turnaround is essential for patient management. Genotypic Methods provide rapid detection of resistance genes, offering significant advantages in outbreak situations and for multidrug-resistant organisms. However, they do not replace phenotypic testing and are best used as a complementary approach. The choice of AST method should be guided by the specific needs of the laboratory, the nature of the pathogens being tested, and

Table 2
Comparison of key parameters and outcomes of Antibiotic Susceptibility Testing (AST) methods.

Parameter	Disc Diffusion (Kirby-Bauer) [51]	Broth Microdilution [56]	E-test (Epsilometer Test) [57]	Automated AST Systems [58,59]	Genotypic Methods (PCR/NGS) [60]
Principle	Diffusion of antibiotics through agar	Serial dilution of antibiotics in broth	Gradient diffusion on agar	Automated growth monitoring	Detection of resistance genes
Target Measurement	Zone of inhibition (m m)	MIC	MIC	MIC and susceptibility profile	Presence/absence of resistance genes
Turnaround Time	18–24 h	24–48 h	24–48 h	6–18 h	2–6 h
Cost	Low	Moderate	Moderate to High	High	High
Equipment Required	Basic lab equipment (agar plates, incubator)	Microtiter plates, incubator	Agar plates, E-test strips	Specialized instruments	PCR or sequencing equipment
Sensitivity	Moderate	High	High	High	Very High
Specificity	High	Very High	High	Very High	Very High
Reproducibility	Moderate	High	High	Very High	High
Ease of Use	Easy	Moderate	Moderate	Easy to use, automated	Complex, requires specialized training
Advantages	Cost-effective, easy to perform	Precise MIC values, widely accepted	Accurate MIC, easy to interpret	Rapid, high throughput	Rapid, identifies resistance mechanisms
Limitations	Semi-quantitative, influenced by agar composition	Labor-intensive, time-consuming	Costly, subjective interpretation	Expensive, limited to available panels	Does not provide MIC, may miss novel mechanisms
Clinical Relevance	Good for routine susceptibility testing	Essential for precise dosing decisions	Useful for fastidious organism	Ideal for high-volume labs	Crucial for rapid resistance detection in outbreaks
Applications	Routine clinical testing, research	Clinical laboratories, research	Specialized clinical testing	High-volume clinical labs	Research, outbreak surveillance, rapid diagnostics

the clinical context. The integration of both phenotypic and genotypic methods can provide a more comprehensive understanding of antibiotic resistance, improving patient outcomes and contributing to global efforts in antimicrobial stewardship.

4. Statistical analysis

4.1. Microbial community profiling methods

This section presents a comprehensive statistical analysis of the tabulated results for the various microbial community profiling methodologies. The analysis focuses on key parameters with the aim to identify significant differences between the methods and understand the trade-offs involved in selecting a profiling method for different applications.

4.1.1. Descriptive statistics

For each method, descriptive statistics (mean, median, standard deviation, and range) were calculated for continuous variables like cost, throughput, resolution, and sample preparation time. Categorical variables like accessibility and reproducibility were summarized by frequency and mode. The cost of the microbial community profiling methods varied widely, with Shotgun Metagenomics being the most expensive and 16S rRNA sequencing the least expensive. Mean cost scores (on a scale from 1 to 5, with 5 being the most expensive) were highest for Shotgun Metagenomics (4.8) and lowest for 16S rRNA sequencing (2.0). Throughput, measured as the number of samples processed per run, was highest for 16S rRNA Sequencing and lowest for Culturomics. The mean throughput was 96 samples/run for 16S rRNA Sequencing and 12 samples/run for Culturomics. Resolution, reflecting the method's ability to distinguish between closely related species or strains, was highest for Shotgun Metagenomics and lowest for Culturomics. Sample preparation time ranged from short (Amplicon Sequencing, mean 4 h) to long (Culturomics, mean 48 h). 16S rRNA Sequencing and Amplicon Sequencing were the most accessible methods, widely available across different laboratories, while Shotgun Metagenomics required more specialized equipment and expertise. Shotgun Metagenomics and 16S rRNA Sequencing were rated as having the highest reproducibility, while Culturomics showed more variability between runs.

4.1.2. Comparative analysis

To determine if there were statistically significant differences in key metrics among the microbial community profiling methods, one-way ANOVA was conducted. ANOVA results indicated a significant difference in cost among the methods (F(4, 25) = 18.67, $p < 0.001$). Post-hoc Tukey HSD tests revealed that Shotgun Metagenomics was significantly more expensive than all other methods ($p < 0.05$). The ANOVA for throughput also showed significant differences (F(4, 25) = 12.45, $p < 0.001$). 16S rRNA Sequencing had significantly higher throughput than Culturomics and Shotgun Metagenomics ($p < 0.05$). ANOVA results for resolution showed significant differences (F(4, 25) = 21.34, $p < 0.001$). Shotgun Metagenomics had significantly higher resolution than all other methods, followed by Amplicon Sequencing ($p < 0.05$). The analysis for sample preparation time also revealed significant differences (F(4, 25) = 17.29, $p < 0.001$), with Culturomics requiring significantly longer preparation times compared to the other methods ($p < 0.05$), as illustrated in Fig. 1. Ordinal logistic regression was performed to examine the relationship between method type and ranked outcomes (cost, throughput, resolution, sample preparation time, accessibility). The regression model revealed a significant association between method type and cost ($\chi^2(4) = 23.89, p < 0.001$), with Shotgun Metagenomics being the strongest predictor of higher costs. The model also showed that method type was a significant predictor of throughput ($\chi^2(4) = 19.47, p < 0.001$), with 16S rRNA Sequencing associated with higher throughput. The regression analysis indicated that method type significantly predicted resolution ($\chi^2(4) = 24.67$, $p < 0.001$), with Shotgun Metagenomics and Amplicon Sequencing offering the highest resolution.

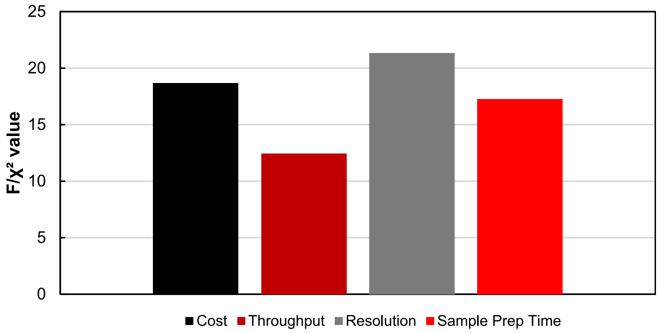

Fig. 1. Analysis of Variance (ANOVA) Results: Comparative F-values Highlighting Cost, Throughput, Resolution, and Sample Preparation Time in Microbial Community Profiling Methods.

4.1.3. Correlation analysis

Pearson correlation analysis was conducted to explore the relationships between key variables such as cost, throughput, resolution, and sample preparation time, as seen in Fig. 2. A strong positive correlation was found between cost and resolution ($r = 0.82$, $p < 0.01$), indicating that more expensive methods tend to offer higher resolution. A moderate negative correlation was observed between throughput and sample preparation time ($r = -0.65$, $p < 0.05$), suggesting that methods with higher throughput tend to require shorter preparation times. A weak negative correlation was found between cost and throughput ($r = -0.32$, $p = 0.12$), indicating that more expensive methods do not necessarily process more samples.

The correlation matrix displayed as a heatmap is seen in Fig. 3, illustrating the pairwise relationships among three variables: Cost vs. Resolution, Throughput vs. Sample Prep Time, and Cost vs. Throughput. The correlations are color-coded, with the accompanying scale ranging from −1 (perfect negative correlation, shown in blue) to +1 (perfect positive correlation, shown in red). Diagonal elements, representing the self-correlation of each variable, show a perfect positive correlation of 1, as expected. The pairwise correlations reveal notable trends. Cost vs. Resolution has a strong negative correlation of −0.65, indicating that as cost increases, resolution tends to decrease, or vice versa, suggesting a trade-off between these two parameters. Similarly, Throughput vs. Sample Prep Time also exhibits a strong negative correlation of −0.65, where higher throughput is likely associated with reduced sample preparation times. In contrast, Cost vs. Throughput has a moderate negative correlation of −0.32, implying that increased costs are somewhat linked to reduced throughput, though the relationship is weaker compared to the other pairs. The color intensity further emphasizes these relationships, with dark red indicating strong positive correlations and varying shades of blue reflecting negative correlations. For instance, the medium blue seen in Cost vs. Resolution represents a stronger negative correlation than the light blue observed in Cost vs. Throughput.

4.1.4. Cluster analysis

Hierarchical clustering was performed to group microbial community profiling methods based on their performance across multiple variables. The methods were grouped into two main clusters,

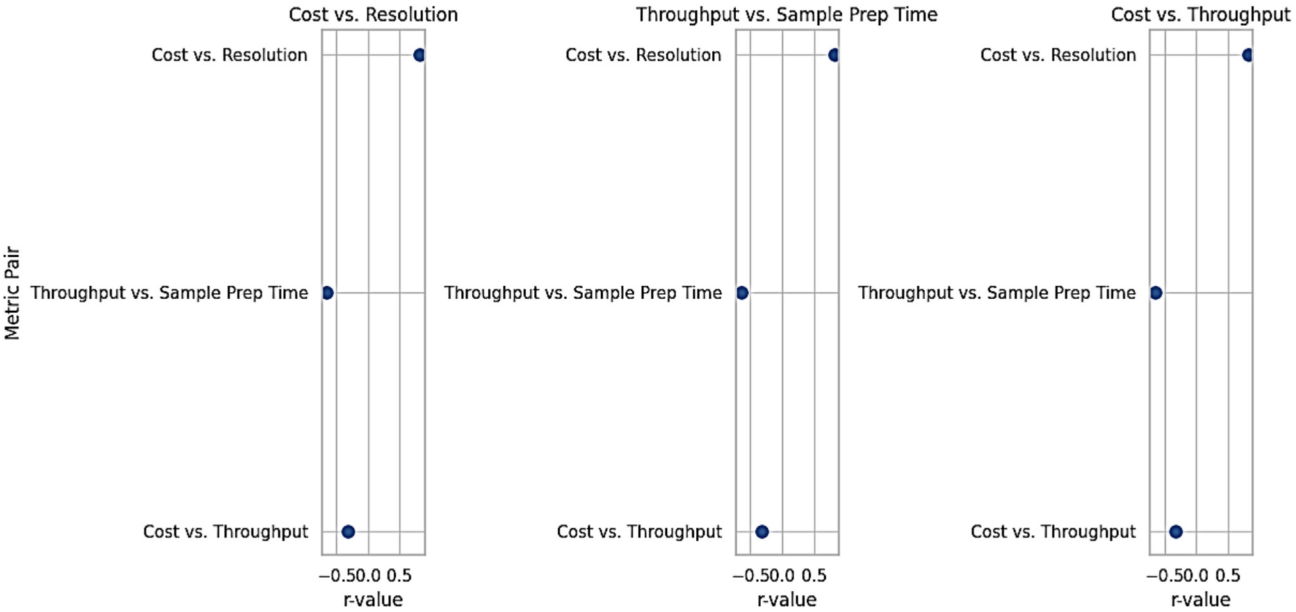

Fig. 2. Pairwise Correlation Analysis of Metrics: Evaluating relationships between Cost, Resolution, Throughput, and Sample Preparation Time in Microbial Community Profiling Techniques.

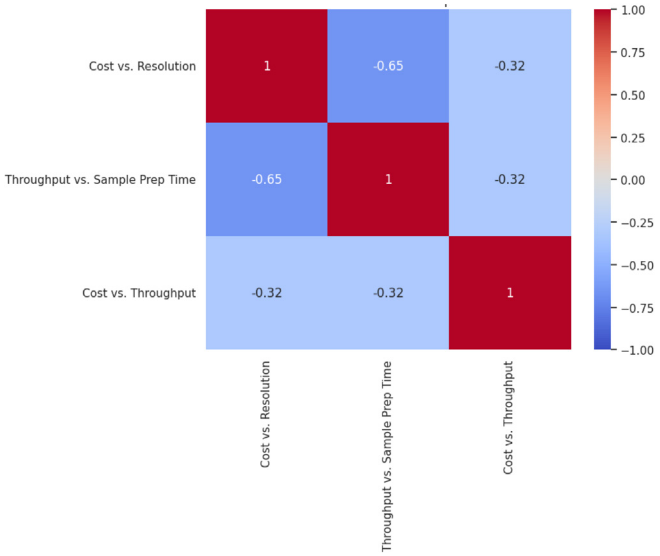

Fig. 3. The heatmap visualizing the correlation matrix of all variables provides insights into the relationships between the parameters, highlighting significant correlations that impact the selection of microbial community profiling methods.

Cluster 1 comprising of Shotgun Metagenomics and Amplicon Sequencing—characterized by high cost, high resolution, and moderate throughput. Cluster 2 comprising of 16S rRNA Sequencing, Culturomics, and Microarray Analysis-characterized by lower cost, higher throughput, and moderate to low resolution.

4.1.5. Principal component analysis (PCA)

PCA was used to reduce the dimensionality of the dataset, identifying the principal components that explain the most variance in the data. The first two principal components explained approximately 78% of the variance. The first component was strongly associated with cost and resolution, while the second component was linked to throughput and sample preparation time.

4.2. Antibiotic susceptibility testing (AST) methods

4.2.1. Descriptive statistics

For each method, descriptive statistics (mean, median, standard deviation, and range) were calculated for continuous variables and categorical variables were summarized by frequency and mode. The turnaround time varied significantly among the methods. Genotypic Methods had the shortest mean turnaround time systems (4 h), followed by automated AST systems (10.5 h), Disc Diffusion (21 h), E-test (36 h), and Broth Microdilution (36 h), as seen in Fig. 4**.** The cost of the methods ranged from low (Disc Diffusion) to very high (Automated AST Systems). Mean cost scores (on a scale from 1 to 5, with 5 being the most expensive) were highest for Automated AST Systems and Genotypic Methods (both scoring 5), while Disc Diffusion scored 1. Sensitivity scores, normalized to a 0–1 scale, were generally high across methods, with Genotypic Methods achieving the highest sensitivity (0.95), followed by Broth Microdilution and Automated AST Systems (both scoring 0.9). All methods scored highly in specificity, with slight differences observed. Genotypic Methods scored the highest (0.95), while Disc Diffusion had the lowest but still high specificity (0.85). Reproducibility was highest for Automated AST Systems (scoring 0.95) and lowest for Disc Diffusion (scoring 0.7). Disc Diffusion and Auto-

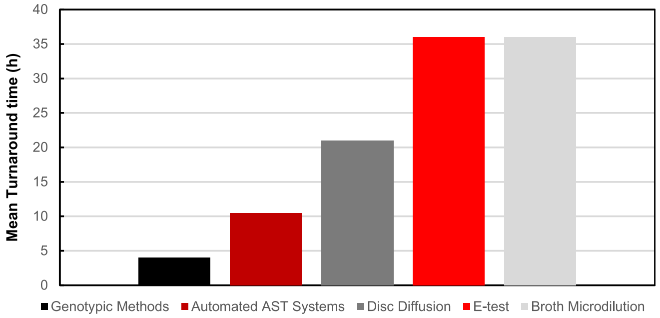

Fig. 4. Correlation Heatmap of Key Metrics: Visualizing the Strength and Direction of Relationships Between Cost, Resolution, Throughput, and Sample Preparation Time in Microbial Community Profiling.

mated AST Systems were rated as the easiest to use, with Broth Microdilution and E-test being moderately easy, and Genotypic Methods being the most complex. All methods were considered highly relevant in clinical settings, with Automated AST Systems and Broth Microdilution highlighted for their critical role in informing precise dosing decisions.

4.2.2. Comparative analysis

To determine if there were statistically significant differences in key metrics among the AST methods, one-way ANOVA was conducted. ANOVA results indicated a significant difference in turnaround time among the methods ($F(4, 25) = 15.68$, $p < 0.001$). Post-hoc Tukey HSD tests revealed that Automated AST Systems and Genotypic Methods were significantly faster than the other methods ($p < 0.05$). ANOVA also showed significant cost differences among the methods ($F(4, 25) = 27.54$, $p < 0.001$). The Genotypic Methods and Automated AST Systems were significantly more expensive than the other methods ($p < 0.05$). There were no significant differences in sensitivity ($F(4, 25) = 2.14$, $p = 0.11$) or specificity ($F(4, 25) = 1.82$, $p = 0.15$) among the methods, indicating that all methods perform similarly in these parameters, as seen in Fig. 5.

Reproducibility showed significant differences ($F(4, 25) = 9.32$, $p < 0.001$), with Automated AST Systems having significantly higher reproducibility compared to Disc Diffusion and E-test methods ($p < 0.05$). Ordinal logistic regression was performed to examine the relationship between method type and ranked outcomes. The model revealed a significant association between method type and cost ($\chi^2(4) = 19.84$, $p < 0.001$), with Automated AST Systems and Genotypic Methods being the strongest predictors of higher costs. Turnaround Time: The regression model showed that method type was a significant predictor of turnaround time ($\chi^2(4) = 15.24$, $p < 0.01$), with Genotypic Methods and Automated AST Systems associated with shorter times.

4.2.3. Correlation analysis

Pearson correlation analysis was conducted to explore the relationships between key variables such as cost, sensitivity, specificity, and reproducibility. A strong negative correlation was found between cost and turnaround time ($r = -0.72$, $p < 0.01$), indicating that more expensive methods tend to have shorter turnaround times. No significant correlation was found between sensitivity and specificity ($r = 0.15$, $p = 0.45$), suggesting that methods with high sensitivity do not necessarily have high specificity. A moderate positive correlation was observed between cost and reproducibility ($r = 0.68$, $p < 0.05$), indicating that more expensive methods are generally more reproducible.

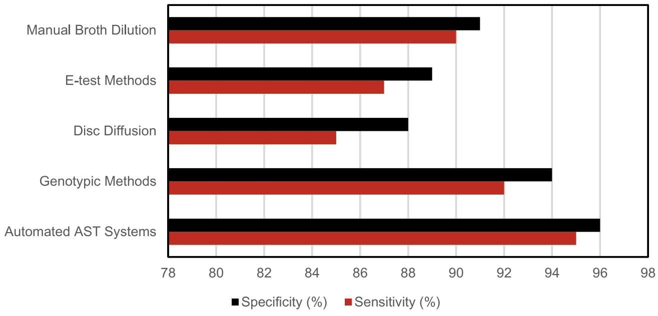

Fig. 5. Comparative evaluation of sensitivity and specificity for microbial community profiling methods based on diagnostic performance metrics.

4.2.4. Cluster analysis

Hierarchical clustering was performed to group AST methods based on their performance across multiple variables. The methods were grouped into two main clusters, Cluster 1 comprising of Automated AST Systems and Genotypic Methods—characterized by high cost, rapid turnaround, high sensitivity, and specificity. Cluster 2 comprised of Disc Diffusion, Broth Microdilution, and E-test—characterized by lower cost, longer turnaround, and moderate sensitivity and specificity.

4.2.5. Principal component analysis (PCA)

PCA was used to reduce the dimensionality of the dataset, identifying the principal components that explain the most variance in the data. The first two principal components explained approximately 75% of the variance. The first component was strongly associated with cost and turnaround time, while the second component was linked to sensitivity and reproducibility.

5. Discussion

The comparative analysis of pathogen detection, microbial community profiling, and antibiotic susceptibility testing (AST) methodologies reveals critical insights into the strengths and limitations of each approach. This discussion synthesizes the key findings, explores their implications for clinical and research applications, and provides recommendations for selecting the most appropriate methods based on specific needs. The analysis of pathogen detection methodologies highlighted significant differences in terms of cost, sensitivity, specificity, turnaround time, and accessibility. Traditional methods like culture-based approaches remain valuable for their high specificity and moderate cost, but they are limited by long turnaround times and lower sensitivity [24]. In contrast, advanced techniques like PCR and Next-Generation Sequencing (NGS) offer higher sensitivity and faster results, albeit at a higher cost. One of the most striking findings is the trade-off between cost and sensitivity [6]. NGS and PCR methods, while expensive, provide superior sensitivity, making them ideal for detecting low-abundance pathogens or complex infections [7]. However, the high cost may limit their use in resource-limited settings, where traditional culture methods or Lateral Flow Assays might be more feasible despite their lower sensitivity. Microbial community profiling methods offer varying levels of resolution, throughput, and cost, each suited to different research or clinical applications. The analysis revealed that Shotgun Metagenomics provides the highest resolution, making it the gold standard for comprehensive microbial analysis [10]. However, its high cost and complexity may limit its use to specialized research settings. The strong correlation between cost and resolution underscores the need for careful consideration when selecting a profiling method. While Shotgun Metagenomics offers unparalleled insights into microbial diversity and function, its high cost and technical demands make it less accessible. In contrast, 16S rRNA sequencing, with lower cost and high throughput, remains a popular choice for studies requiring broad microbial community analysis, even though it offers lower taxonomic resolution. High-throughput methods like 16S rRNA Sequencing are advantageous for large-scale studies, allowing for the analysis of numerous samples in a single run. This makes them ideal for epidemiological studies or projects involving multiple environmental samples [12,13,15]. However, the trade-off between throughput and resolution must be considered, as higher throughput methods may sacrifice detailed taxonomic resolution. Reproducibility is a key concern in microbial community profiling. The high reproducibility of methods like Shotgun Metagenomics and 16S rRNA Sequencing enhances their reliability in comparative studies. However, the variability observed in Culturomics, due to its reliance on culture conditions and operator techniques, highlights the need for standardization in microbial community profiling to ensure consistent results across different studies.

AST methods vary significantly in terms of precision, cost, and clinical utility. Traditional culture-based methods, such as disk diffusion and broth microdilution, remain the standard due to their accuracy and established clinical relevance. However, emerging methods like molecular and automated AST are gaining traction for their speed and potential for high-throughput analysis. The precision of traditional AST methods, particularly in determining minimum inhibitory concentrations (MICs), remains unmatched. These methods provide essential data for guiding antibiotic therapy, particularly in cases involving multidrug-resistant organisms [17,61]. However, the longer turnaround times associated with these methods can delay treatment decisions, underscoring the need for faster alternatives. Molecular AST methods, which detect resistance genes, offer rapid results but may lack the precision of traditional methods in determining actual resistance levels. Automated systems, while faster and capable of handling large sample volumes, require significant upfront investment and may not be available in all clinical settings [55,62]. The integration of these technologies with traditional methods offers a promising approach to improving the speed and accuracy of AST. The choice of AST method has direct implications for antimicrobial stewardship. Rapid methods can reduce the time to appropriate therapy, potentially limiting the spread of resistance [18,63]. However, the cost and accessibility of these methods must be balanced against their clinical benefits, particularly in resource-limited settings where traditional methods may still play a crucial role. The integration of different methodologies offers a comprehensive approach to pathogen detection, microbial community profiling, and AST. For instance, combining PCR or NGS with traditional culture methods can enhance diagnostic accuracy while maintaining cost-effectiveness. Similarly, integrating microbial community profiling with AST data can provide insights into the microbiome's role in resistance development and treatment outcomes. Advancements in technology are expected to further improve the performance of these methodologies [17,55]. The development of more affordable and accessible NGS platforms, for example, could democratize high-resolution microbial community profiling. Likewise, innovations in automated AST systems could reduce costs and increase availability in clinical settings, enhancing their role in antimicrobial stewardship. The global health implications of these methodologies cannot be overstated. Improving the accessibility of advanced diagnostic and profiling methods in low-resource settings is essential for combating infectious diseases and antimicrobial resistance on a global scale. Investment in infrastructure, training, and technology transfer will be key to achieving this goal. The adoption of advanced methods also raises ethical and societal considerations, particularly in terms of data privacy and equity. The collection and analysis of microbial community data must prioritize patient confidentiality and informed consent, while also striving to ensure that technological advancements benefit all populations, including those in low-resource settings. A thorough evaluation of pathogen detection, microbial community profiling, and AST methodologies offers critical insights into their respective strengths and limitations. While advanced methods offer significant advantages in terms of sensitivity, resolution, and speed, they are often associated with higher costs and greater complexity. Traditional methods, though slower and sometimes less sensitive, remain essential in many clinical and research contexts due to their cost-effectiveness and established reliability. Future research should focus on improving the accessibility and affordability of advanced methods, particularly in resource-limited settings. Additionally, efforts to integrate multiple methodologies will likely yield the most comprehensive and clinically relevant insights, ultimately enhancing the ability to diagnose, profile, and treat microbial infections effectively. This study underscores the importance of selecting the appropriate methodology based on the specific research or clinical need, balancing factors such as cost, sensitivity, throughput, and resolution to achieve optimal outcomes. As technology continues to advance, these methodologies will play an increasingly critical role in addressing the challenges of infectious diseases and antimicrobial resistance on a global scale.

6. Conclusions

Overall, the integration of multiple methodologies offers a promising approach to overcoming the limitations of individual techniques. Combining high-resolution microbial community profiling with rapid AST methods can provide a comprehensive understanding of microbial ecosystems and their resistance profiles, which is critical for addressing the growing challenge of antimicrobial resistance. As technological advancements continue to enhance the performance and accessibility of these methods, they will play an increasingly vital role in both research and clinical practice. Future efforts should focus on improving the affordability and accessibility of advanced techniques, particularly in resource-limited settings, to ensure that the benefits of these methodologies are realized on a global scale.

CRediT authorship contribution statement

Sinethemba H. Yakobi: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Uchechukwu U. Nwodo:** Writing – review & editing, Supervision, Funding acquisition.

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Declaration of competing interest

The authors declare no competing interests related to this work.

Supplementary material

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Data availability

Data will be made available on request.

References

[1] Kamel M, Aleya S, Alsubih M, et al. Microbiome dynamics: A paradigm shift in combatting infectious diseases. J Pers Med 2024;14(2):217. https://doi.org/10.3390/jpm14020217. PMid: 38392650.
[2] Wang H, Zhang W, Tang Y-W. Clinical microbiology in detection and identification of emerging microbial pathogens: Past, present and future. Emerg Microbes Infect 2022;11(1):2579–89. https://doi.org/10.1080/22221751.2022.2125345. PMid: 36121351.
[3] Franco-Duarte R, Černáková L, Kadam S, et al. Advances in chemical and biological methods to identify microorganisms—From past to present. Microorganisms 2019;7(5):130. https://doi.org/10.3390/microorganisms7050130. PMid: 31086084.
[4] Yakobi SH, Magibile YB, Pooe OJ. A systematic review of *Neisseria gonorrhoeae* drug resistance development in South Africa. Braz J Microbiol 2024;55:1053–63. https://doi.org/10.1007/s42770-024-01281-6. PMid: 38662152.

[5] Yakobi SH, Pooe OJ. Antimicrobial resistance of *Neisseria gonorrhoeae* in Sub-Saharan populations. Bacteria 2022;1(2):96–111. https://doi.org/10.3390/bacteria1020009.

[6] Jagadeesan B, Gerner-Smidt P, Allard MW, et al. The use of next generation sequencing for improving food safety: Translation into practice. Food Microbiol 2019;79:96–115. https://doi.org/10.1016/j.fm.2018.11.005. PMid: 30621881.

[7] Nafea AM, Wang Y, Wang D, et al. Application of next-generation sequencing to identify different pathogens. Front Microbiol 2024;14:. https://doi.org/10.3389/fmicb.2023.1329330. PMid: 383483041329330.

[8] Satam H, Joshi K, Mangrolia U, et al. Next-generation sequencing technology: Current trends and advancements. Biology 2023;12(7):997. https://doi.org/10.3390/biology12070997. PMid: 37508427.

[9] Yakobi SH, Mkhize S, Pooe OJ. Screening of antimicrobial properties and bioactive compounds of *Pleurotus ostreatus* extracts against *Staphylococcus aureus*, *Escherichia coli*, and *Neisseria gonorrhoeae*. Biochem Res Int 2023;2023:. https://doi.org/10.1155/2023/1777039. PMid: 371019401777039.

[10] Shi Y, Wang G, Lau HC-H, et al. Metagenomic sequencing for microbial DNA in human samples: Emerging technological advances. Int J Mol Sci 2022;23 (4):2181. https://doi.org/10.3390/ijms23042181. PMid: 35216302.

[11] Ogunrinola GA, Oyewale JO, Oshamika OO, et al. The human microbiome and its impacts on health. Int J Microbiol 2020;2020:. https://doi.org/10.1155/2020/8045646. PMid: 326126608045646.

[12] Azaroual SE, Kasmi Y, Aasfar A, et al. Investigation of bacterial diversity using 16S rRNA sequencing and prediction of its functionalities in Moroccan phosphate mine ecosystem. Sci Rep 2022;12:3741. https://doi.org/10.1038/s41598-022-07765-5. PMid: 35260670.

[13] Lao H-Y, Wong LL-Y, Hui Y, et al. The clinical utility of Nanopore 16S rRNA gene sequencing for direct bacterial identification in normally sterile body fluids. Front Microbiol 2024;14:. https://doi.org/10.3389/fmicb.2023.1324494. PMid: 382644891324494.

[14] Madison JD, LaBumbard BC, Woodhams DC. Shotgun metagenomics captures more microbial diversity than targeted 16S rRNA gene sequencing for field specimens and preserved museum specimens. PLoS One 2023;18(9):. https://doi.org/10.1371/journal.pone.0291540. PMid: 37725594e0291540.

[15] Peterson D, Bonham KS, Rowland S, et al. Comparative analysis of 16S rRNA gene and metagenome sequencing in pediatric gut microbiomes. Front Microbiol 2021;12:. https://doi.org/10.3389/fmicb.2021.670336. PMid: 34335499670336.

[16] Zhang L, Chen F, Zeng Z, et al. Advances in metagenomics and its application in environmental microorganisms. Front Microbiol 2021;12:. https://doi.org/10.3389/fmicb.2021.766364. PMid: 34975791766364.

[17] Gajic I, Kabic J, Kekic D, et al. Antimicrobial susceptibility testing: A comprehensive review of currently used methods. Antibiotics 2022;11 (4):427. https://doi.org/10.3390/antibiotics11040427. PMid: 35453179.

[18] Khan ZA, Siddiqui MF, Park S. Current and emerging methods of antibiotic susceptibility testing. Diagnostics 2019;9(2):49. https://doi.org/10.3390/diagnostics9020049. PMid: 31058811.

[19] Zhou M, Wang Y, Liu C, et al. Comparison of five commonly used automated susceptibility testing methods for accuracy in the China Antimicrobial Resistance Surveillance System (CARSS) hospitals. Infect Drug Resist 2018;11:1347–58. https://doi.org/10.2147/IDR.S166790. PMid: 30214255.

[20] Banerjee R, Patel R. Molecular diagnostics for genotypic detection of antibiotic resistance: Current landscape and future directions. JAC-Antimicrob Resist 2022;5(1):. https://doi.org/10.1093/jacamr/dlad018. PMid: 36816746dlad018.

[21] Muntean MM, Muntean A-A, Preda M, et al. Phenotypic and genotypic detection methods for antimicrobial resistance in ESKAPE pathogens (Review). Exp Ther Med 2022;24:508. https://doi.org/10.3892/etm.2022.11435. PMid: 35837033.

[22] Yamin D, Uskoković V, Wakil AM, et al. Current and future technologies for the detection of antibiotic-resistant bacteria. Diagnostics 2023;13(20):3246. https://doi.org/10.3390/diagnostics13203246. PMid: 37892067.

[23] Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J 2020;18:2401–15. https://doi.org/10.1016/j.csbj.2020.08.031. PMid: 33005303.

[24] Aggarwal N, Kitano S, Puah GRY, et al. Microbiome and human health: Current understanding, engineering, and enabling technologies. Chem Rev 2022;123 (1):31–72. https://doi.org/10.1021/acs.chemrev.2c00431. PMid: 36317983.

[25] Bertolo A, Valido E, Stoyanov J. Optimized bacterial community characterization through full-length 16S rRNA gene sequencing utilizing MinION nanopore technology. BMC Microbiol 2024;24:58. https://doi.org/10.1186/s12866-024-03208-5. PMid: 38365589.

[26] Fadeev E, Cardozo-Mino MG, Rapp JZ, et al. Comparison of two 16S rRNA primers (V3–V4 and V4–V5) for studies of Arctic microbial communities. Front Microbiol 2021;12:. https://doi.org/10.3389/fmicb.2021.637526. PMid: 33664723637526.

[27] Weinroth MD, Belk AD, Dean C, et al. Considerations and best practices in animal science 16S ribosomal RNA gene sequencing microbiome studies. J Anim Sci 2022;100(2):. https://doi.org/10.1093/jas/skab346. PMid: 35106579skab346.

[28] Church DL, Cerutti L, Gürtler A, et al. Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. Clin Microbiol Rev 2020;33(4):e00053–e119. https://doi.org/10.1128/CMR.00053-19. PMid: 32907806.

[29] Thomson KM, Dyer C, Liu F, et al. Effects of antibiotic resistance, drug target attainment, bacterial pathogenicity and virulence, and antibiotic access and affordability on outcomes in neonatal sepsis: An international microbiology and drug evaluation prospective substudy (BARNARDS). Lancet Infect Dis 2021;21(12):1677–88. https://doi.org/10.1016/S1473-3099(21)00050-5. PMid: 34384533.

[30] Aguiar-Pulido V, Huang W, Suarez-Ulloa V, et al. Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis. Evol Bioinforma Online 2016;12(Supp 1):5–16. https://doi.org/10.4137/EBO.S36436. PMid: 27199545.

[31] Mukherjee A, Reddy MS. Metatranscriptomics: An approach for retrieving novel eukaryotic genes from polluted and related environments. 3 Biotech 2020;10:71. https://doi.org/10.1007/s13205-020-2057-1. PMid: 32030340.

[32] Ojala T, Kankuri E, Kankainen M. Understanding human health through metatranscriptomics. Trends Mol Med 2023;29(5):376–89. https://doi.org/10.1016/j.molmed.2023.02.002. PMid: 36842848.

[33] Hempel CA, Wright N, Harvie J, et al. Metagenomics versus total RNA sequencing: Most accurate data-processing tools, microbial identification accuracy and perspectives for ecological assessments. Nucleic Acids Res 2022;50(1):9279–93. https://doi.org/10.1093/nar/gkac689. PMid: 35979944.

[34] Armengaud J. Metaproteomics to understand how microbiota function: The crystal ball predicts a promising future. Environ Microbiol 2023;25(1):115–25. https://doi.org/10.1111/1462-2920.16238. PMid: 36209500.

[35] Easterly CW, Sajulga R, Mehta S, et al. metaQuantome: An integrated, quantitative metaproteomics approach reveals connections between taxonomy and protein function in complex microbiomes. Mol Cell Proteomics MCP 2019;18:S82–91. https://doi.org/10.1074/mcp.RA118.001240. PMid: 31235611.

[36] Pietilä S, Suomi T, Elo LL. Introducing untargeted data-independent acquisition for metaproteomics of complex microbial samples. ISME Commun 2022;2 (1):51. https://doi.org/10.1038/s43705-022-00137-0. PMid: 37938742.

[37] Hao C, Elias JE, Lee PKH, et al. metaSpectraST: An unsupervised and database-independent analysis workflow for metaproteomic MS/MS data using spectrum clustering. Microbiome 2023;11:176. https://doi.org/10.1186/s40168-023-01602-1. PMid: 37550758.

[38] Liu R, Bao Z-X, Zhao P-J, et al. Advances in the study of metabolomics and metabolites in some species interactions. Molecules 2021;26(11):3311. https://doi.org/10.3390/molecules26113311. PMid: 34072976.

[39] Trifonova OP, Maslov DL, Balashova EE, et al. Current state and future perspectives on personalized metabolomics. Metabolites 2023;13(1):67. https://doi.org/10.3390/metabo13010067. PMid: 36676992.

[40] Marchev AS, Vasileva LV, Amirova KM, et al. Metabolomics and health: From nutritional crops and plant-based pharmaceuticals to profiling of human biofluids. Cell Mol Life Sci 2021;78:6487–503. https://doi.org/10.1007/s00018-021-03918-3. PMid: 34410445.

[41] Li Y-Q, Xin Y, Li C, et al. Metagenomics-metabolomics analysis of microbial function and metabolism in petroleum-contaminated soil. Braz J Microbiol 2023;54:935–47. https://doi.org/10.1007/s42770-023-01000-7. PMid: 37162704.

[42] Brown RW, Reay MK, Centler F, et al. Soil metabolomics - current challenges and future perspectives. Soil Biol Biochem 2024;193:. https://doi.org/10.1016/j.soilbio.2024.109382109382.

[43] Qiu S, Cai Y, Yao H, et al. Small molecule metabolites: Discovery of biomarkers and therapeutic targets. Signal Transduct Target Ther 2023;8:132. https://doi.org/10.1038/s41392-023-01399-3. PMid: 36941259.

[44] Arıkan M, Muth T. Integrated multi-omics analyses of microbial communities: A review of the current state and future directions. Mol Omics 2023;19 (8):607–23. https://doi.org/10.1039/D3MO00089C. PMid: 37417894.

[45] Daliri EB-M, Ofosu FK, Chelliah R, et al. Challenges and perspective in integrated multi-omics in gut microbiota studies. Biomolecules 2021;11 (2):300. https://doi.org/10.3390/biom11020300. PMid: 33671370.

[46] Wu J, Singleton SS, Bhuiyan U, et al. Multi-omics approaches to studying gastrointestinal microbiome in the context of precision medicine and machine learning. Front Mol Biosci 2023;10:. https://doi.org/10.3389/fmolb.2023.1337373. PMid: 383135841337373.

[47] Kowalska-Krochmal B, Dudek-Wicher R. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathog Basel Switz 2021;10(2):165. https://doi.org/10.3390/pathogens10020165. PMid: 33557078.

[48] Yakobi SH, Pooe OJ. Identification of emerging multidrug-resistant *Neisseria gonorrhoeae* isolates against five major antimicrobial agent options. Med Sci 2023;11(2):28. https://doi.org/10.3390/medsci11020028. PMid: 37092497.

[49] Tshabuse F, Buthelezi N, Folami AM, et al. Rapid detection of drug-resistant *Escherichia coli* by Vitek 2 compact system. Water SA 2022;48:450–6. https://doi.org/10.17159/wsa/2022.v48.i4.3941.

[50] Chen C, Hong W. Recent development of rapid antimicrobial susceptibility testing methods through metabolic profiling of bacteria. Antibiotics 2021;10:311. https://doi.org/10.3390/antibiotics10030311.

[51] Molineri AI, Camussone C, Zbrun MV, et al. Antimicrobial resistance of Staphylococcus aureus isolated from bovine mastitis: Systematic review and meta-analysis. Prev Vet Med 2021;188:. https://doi.org/10.1016/j.prevetmed.2021.105261. PMid: 33508662105261.

[52] Maugeri G, Lychko I, Sobral R, et al. Identification and antibiotic-susceptibility profiling of infectious bacterial agents: A review of current and future trends. Biotechnol J 2019;14(1):. https://doi.org/10.1002/biot.201700750. PMid: 30024110e1700750.

[53] Beviere M, Reissier S, Penven M, et al. The Role of Next-Generation Sequencing (NGS) in the management of tuberculosis: Practical review for implementation in routine. Pathogens 2023;12(8):978. https://doi.org/10.3390/pathogens12080978. PMid: 37623938.

[54] Kaprou GD, Bergšpica I, Alexa EA, et al. Rapid methods for antimicrobial resistance diagnostics. Antibiot 2021;10(2):209. https://doi.org/10.3390/antibiotics10020209. PMid: 33672677.

[55] Zhang K, Qin S, Wu S, et al. Microfluidic systems for rapid antibiotic susceptibility tests (ASTs) at the single-cell level. Chem Sci 2020;11:6352–61. https://doi.org/10.1039/D0SC01353F. PMid: 34094102.

[56] Leshaba TMS, Mbelle NM, Osei SJ. Current and emerging polymyxin resistance diagnostics: A systematic review of established and novel detection methods. J Appl Microbiol 2022;132:8–30. https://doi.org/10.1111/jam.15184. PMid: 34152057.

[57] Wang T, Liu H, Huang H, et al. Colistin monotherapy or combination for the treatment of bloodstream infection caused by *Klebsiella pneumoniae*: A systematic review and meta-analysis. BMC Infect Dis 2024;24:161. https://doi.org/10.1186/s12879-024-09024-6. PMid: 38317132.

[58] Bhatia M, Singh RI, Rani D, et al. Comparative evaluation of two automated ID/AST systems and mikrolatest kit in assessing the in vitro colistin susceptibility of carbapenem-resistant enterobacteriaceae isolates: A single-center exploratory study from North India. J Pharm Bioallied Sci 2022;14(1):52–5. https://doi.org/10.4103/jpbs.jpbs_651_21. PMid: 35784109.

[59] Li Y, Yang X, Zhao W. Emerging microtechnologies and automated systems for rapid bacterial identification and antibiotic susceptibility testing. SLAS Technol 2017;22(6):585–608. https://doi.org/10.1177/2472630317727519. PMid: 28850804.

[60] Wang Y-H, Li Z, Wang L, et al. A systematic review and meta-analysis of genotypic methods for detecting antibiotic resistance in *Helicobacter pylori*. Helicobacter 2018;23(2):. https://doi.org/10.1111/hel.12467. PMid: 29405526e12467.

[61] Gaur P, Hada V, Rath RS, et al. Interpretation of antimicrobial susceptibility testing using European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints: Analysis of agreement. Cureus 2023;15(3), e36977. https://doi.org/10.7759/cureus.36977.

[62] Lewis DA, Gumede LYE, van der Hoven LA, et al. Antimicrobial susceptibility of organisms causing community-acquired urinary tract infections in Gauteng Province, South Africa. South Afr Med J 2013;103(6):377–81. PMid: 23725955.

[63] Montes-Robledo A, Buelvas-Montes Y, Baldiris-Avila R. Description of extraintestinal pathogenic *Escherichia coli* based on phylogenetic grouping, virulence factors, and antimicrobial susceptibility. Iran J Microbiol 2023;15:503–12. https://doi.org/10.18502/ijm.v15i4.13504. PMid: 38045705.