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Identification test of bacterial species and resistance genes from infectious patients using MDR direct flow chip molecular technique compared to conventional techniques

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ABSTRACT

Introduction: Antimicrobial resistance poses a threat to global public health by increasing morbidity and mortality. While resistance development is a natural biological process, antibiotic use has accelerated its rise resistance. This study aimed to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the *MDR Direct Flow Chip Kit* to detect 5 bacterial species and a total of 55 resistance gene markers.

Methods: The research employed a cross-sectional design, analyzing positive samples determined by the VITEK® 2 system from various specimens, including sputum, pus, and blood. These specimens were collected from infected patients at the FKUI Clinical Microbiology Laboratory in 2023. They were stored in Eppendorf tubes with TSB and Glycerol fluids, then cultured on Nutrient Agar Media and incubated overnight. HybriSpot® will analyze the increasing isolates using the MDR Direct Flow Chip, an in vitro diagnostic kit that quickly detects multidrug-resistant bacteria. This kit employs multiplex PCR to identify five bacterial species—Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Acinetobacter baumannii—as well as 55 resistance gene markers. These markers include the primary enzymatic mechanisms associated with nine different antibiotic classes.

Result: A total of 40 samples were analyzed with HybriSpot® using the *MDR Direct Flow Chip*, which identified 5 genus species and detected 31 resistance genes. The same samples were also tested with the VITEK® 2 system, which identified the same 5 genus species and provided antibiotic susceptibility results. The overall sensitivity was 80%, specificity was 94.2%, and conformity was 94.68%.

Conclusion: The *MDR Direct Flow Chip* assay is a promising method for identifying pathogens in various specimen cultures of infected patients and can aid antimicrobial stewardship programs in hospitals.

Keywords: antimicrobial resistance, hybriSpot®, VITEK® 2.
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INTRODUCTION

The resistance of different microbial species (infectious agents) to various antimicrobial drugs has become a global public health concern. As new resistance mechanisms emerge and the effectiveness of standard treatments declines, it leads to treatment failures, prolonged illnesses, and increased mortality. Nearly all infectious agents, including bacteria, fungi, viruses, and parasites, have developed significant multidrug resistance.[1–3] Multidrug resistance arises from the improper use of antimicrobial drugs, poor sanitation, incorrect food handling, and inadequate infection prevention, all of which promote the emergence and spread of resistance. As healthcare technology advances, new diagnostic techniques are also being developed. One notable molecular method is the *MDR Direct Flow Chip*.[1]

MDR Direct Flow Chip is a multiplex PCR-based diagnostic method that works automatically and can detect pathogenic bacteria and antibiotic resistance genes within 2 hours.[4] The *MDR Direct Flow Chip* method detects the five most common pathogenic bacteria such as Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Acinetobacter baumannii, along with 55 antibiotic resistance gene markers.[5] *MDR Direct Flow Chip* is an automated multiplex PCR-based diagnostic method that can detect pathogenic bacteria and their antibiotic sensitivity pattern in just 2 hours.[4] With these technologies, examinations can be carried out faster and more accurately, even for the examination of large numbers of specimens.[5]

METHODS

The research employed a cross-sectional study design with positive samples confirmed by VITEK® 2 testing. These samples included sputum, pus, blood, bronchial washing, and urine collected from infected patients examined at the FKUI Clinical Microbiology Laboratory in 2023. Specimens were collected in Eppendorf tubes containing TSB and

Glycerol fluids, then culture procedures were carried out on Nutrient Agar Media and incubated overnight. The growing isolates will be examined by HybriSpot® using the *MDR Direct Flow Chip*. The results are analyzed automatically with hybriSoft™ software.

RESULTS

A total of 40 samples were tested with HybriSpot® using the *MDR Direct Flow Chip*, identifying 5 genera of species and detecting 31 resistance genes (see **Figure 1**). An examination was also carried out using the VITEK® 2 system, which identified 5 genus species and antibiotic sensitivity test results (**Figure 2**). In the *MDR Direct Flow Chip* examination, *Acinetobacter baumannii* was identified with a total of 8, *Escherichia coli* 19, *Klebsiella pneumoniae* 10, *Pseudomonas aeruginosa* 3, and *Staphylococcus aureus* 1 were identified. There were several samples that were not identified as pathogens. In the VITEK® 2 examination, the pathogen identification was obtained for *Acinetobacter baumannii* 1, *Escherichia coli* 13, *Klebsiella pneumoniae ssd pneumoniae* 10, *Pseudomonas aeruginosa* 2, and *Staphylococcus aureus* 4. All samples obtained pathogen identification using the VITEK® 2 system. Sensitivity, specificity, and conformity were obtained as 80%, 94.2%, and 94.68%.

In the examination using Hybrispot®, *Acinetobacter baumannii* bacteria were found to have resistance genes in the form of Carbapenemase OXA51, Sulfonamide resistance gene (Sul-1), Aminoglycoside resistance gene (aac6-lb), and Chloramphenicol resistance gene (catB3). In *Escherichia coli*, the sulfonamide resistance gene (sul-2) was found. In other *Escherichia coli* bacteria, resistance genes were found in the form of Sulfonamide resistance gene (sul-1), Sulfonamide resistance gene (sul-2), Quinolones or fluoroquinolones resistance gene (qnrS), Olaquindox resistance gene (oqxA), and Chloramphenicol resistance gene (catB3). In *Klebsiella pneumoniae* and *Escherichia coli* bacteria obtained from Hybrispot®, resistance genes were found in the form of B-lactamase SHV, Sulfonamide resistance gene (sul-1), Quinolones or fluoroquinolones resistance gene (qnrB),

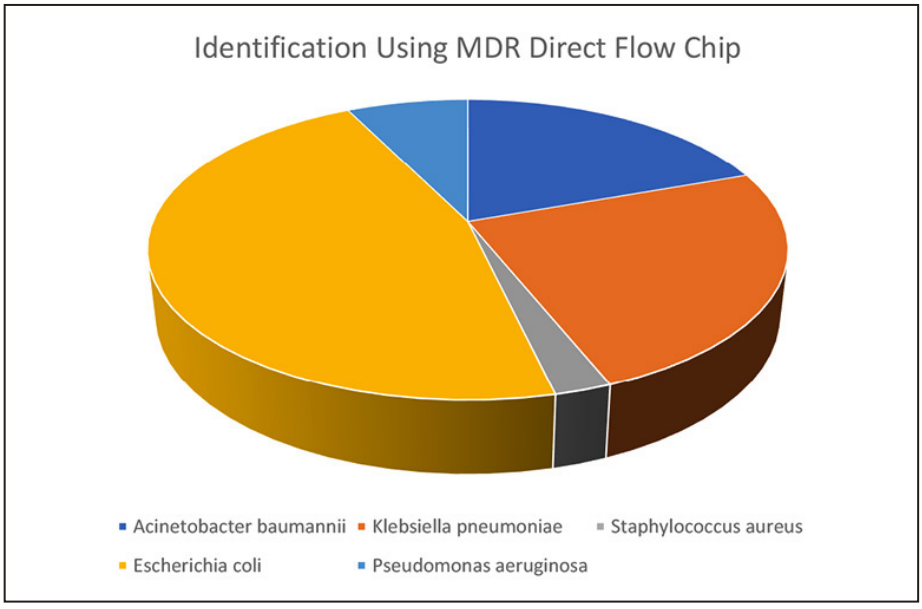

Figure 1. Identification of bacteria using the MDR *direct flow chip*.

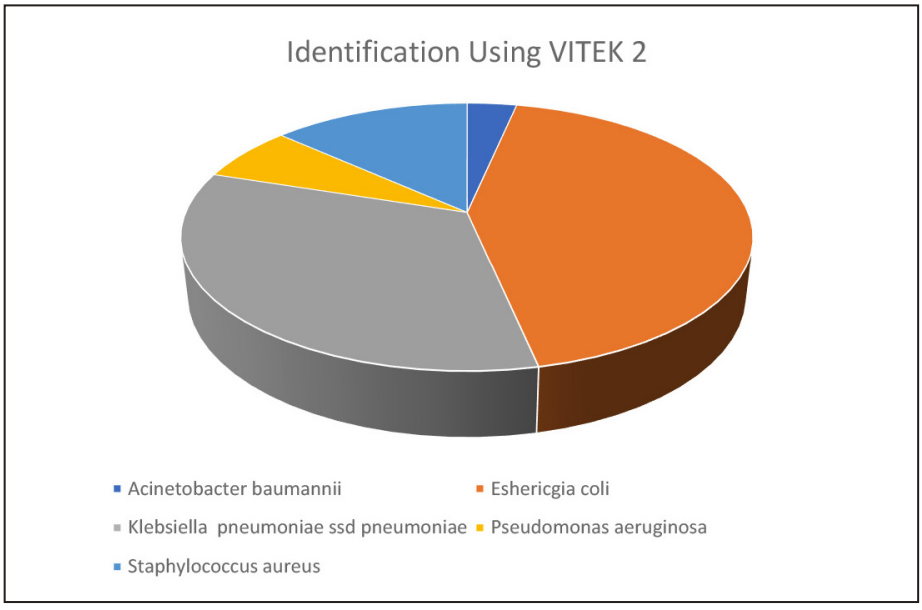

Figure 2. Identification of bacteria using VITEK 2.

Quinolones or fluoroquinolones resistance gene (qnrS), Olaquindox resistance gene (oqxA), Olaquindox resistance gene(oqxB), and Chloramphenicol resistance gene (catB3). These results were obtained from 8 specimens collected.

In *Pseudomonas aeruginosa* and *Escherichia coli* bacteria obtained from Hybrispot®, resistance genes were found in the form of Carbapenemase VIM, Sulfonamide resistance gene (sul-1), Sulfonamide resistance gene (sul-2), Fluoroquinolones resistance (mut. gyrP-T831), Quinolones or fluoroquinolones resistance gene (qnrS), and Olaquindox resistance gene (oqxA). These results were obtained on 1 specimen. In the *Acinetobacter baumannii* bacteria obtained, resistance genes were found in the form of Carbapenemase OXA51_like, Sulfonamides resistance gene (sul-1), Aminoglycosides resistance gene (aac(6')-lb), and Chloramphenicol resistance gene (catB3). In *Acinetobacter baumannii* and *Escherichia coli*, resistance genes were found in the form of Sulfonamide resistance gene (sul-1), Sulfonamide resistance gene (sul-2), Quinolones or fluoroquinolones resistance gene (qnrB), Quinolones or fluoroquinolones resistance gene (qnrS),

Olaquindox resistance gene (oqxA), Olaquindox resistance gene(oqxB), and Chloramphenicol resistance gene (catB3). These results were obtained in 2 specimens. From 10 specimens collected, no bacterial identification was found using Hybrispot®. In *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Escherichia coli*, resistance genes were found in the form of Sulfonamide resistance gene (sul-1), Sulfonamide resistance gene (sul-2), Low-level fluoroquinolones resistence (mut.gyrE-S83L), Quinolones or fluoroquinolones resistance gene (qnrB), Quinolones or fluoroquinolones resistance gene (qnrS), and Chloramphenicol resistance gene (catB3). Bacterial species and their resistance genes in Hybrispot® are shown in **Table 1**.

Table 1. Bacterial species and their resistance genes in Hybrispot®

Type of Bacteria	Gene Resistance in Hybrispot®
Acinetobacter baumannii	Carbapenemase OXA51, Sulfonamide resistance gene (Sul-1), Aminoglycoside resistance gene (aac6-lb), Chloramphenicol resistance gene (catB3).
Escherichia coli	Sulfonamide resistance gene (sul-1), Sulfonamide resistance gene (sul-2), Quinolones or fluoroquinolones resistance gene (qnrS), Olaquindox resistance gene (oqxA), Chloramphenicol resistance gene (catB3)
Klebsiella pneumoniae, Escherichia coli	B-lactamase SHV, Sulfonamide resistance gene (sul-1), Quinolones or fluoroquinolones resistance gene (qnrB), Quinolones or fluoroquinolones resistance gene (qnrS), Olaquindox resistance gene (oqxA), Olaquindox resistance gene(oqxB), Chloramphenicol resistance gene (catB3).
Pseudomonas aeruginosa, *Escherichia coli*	Carbapenemase VIM, Sulfonamide resistance gene (sul-1), Sulfonamide resistance gene (sul-2), Fluoroquinolones resistance (mut. gyrP-T831), Quinolones or fluoroquinolones resistance gene (qnrS), Olaquindox resistance gene (oqxA).
Acinetobacter baumannii, Escherichia coli	of Sulfonamide resistance gene (sul-1), Sulfonamide resistance gene (sul-2), Quinolones or fluoroquinolones resistance gene (qnrB), Quinolones or fluoroquinolones resistance gene (qnrS), Olaquindox resistance gene (oqxA), Olaquindox resistance gene(oqxB), Chloramphenicol resistance gene (catB3).
Staphylococcus aureus, *Acinetobacter baumannii*, *Escherichia coli*	Sulfonamide resistance gene (sul-1), Sulfonamide resistance gene (sul-2), Low-level fluoroquinolones resistence (mut.gyrE-S83L), Quinolones or fluoroquinolones resistance gene (qnrB), Quinolones or fluoroquinolones resistance gene (qnrS), Chloramphenicol resistance gene (catB3)

DISCUSSION

Antibiotic resistance (AMR) has become a major public health issue in the 21st century, jeopardizing the ability to prevent and treat infections caused by bacteria that no longer respond to antibiotics. The challenge of AMR is especially urgent concerning bacterial resistance. Over time, bacteria responsible for various infections have evolved resistance to every new antibiotic introduced to the market.[6] Early detection is crucial; internal phenotypic tests are time-consuming, whereas PCR methods are costly and limited to specific genes. The new DNA microarray, used with multiplex PCR, enables earlier detection. The *MDR Direct Flow Chip* is a novel microarray-based assay designed for in vitro identification of resistance genes.[7]

The *MDR Direct Flow Chip* technique involves the simultaneous amplification of *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii*, and 56 resistance genes through multiplex PCR from cell extracts, bacterial colonies, and/or blood cultures. This is followed by membrane hybridization with specific DNA probes using DNA flow technology for HybriSpot® platform.[8] This diagnostic method works automatically; it can detect pathogenic bacteria and antibiotic resistance genes within 2 hours.[4] The disadvantage of the *MDR Direct Flow Chip* technique are the identification of bacteria can not reach the species level identification and can only identify 5 bacterial species. However, the advantage of the *MDR Direct Flow Chip* technique is show the presence of resistance gene markers that provide an indication or information about the infection that has occurred from bacteria that are resistant to several antibiotics.[9]

The VITEK® 2 system is an automated platform for identifying microbes and testing their antibiotic susceptibility after a standard inoculum is prepared. Identification results are typically available within 3 hours, while susceptibility testing takes about 18 hours. Due to the urgent need to accelerate these processes, especially in positive blood cultures, recent developments have enabled faster identification, often within 1 hour.[10]

In the previous study by Villodres et al., the *MDR Direct Flow Chip* method was used to identify gene resistance in clinical isolates. A total of 92 isolates, comprising various bacterial species, were tested. This technique successfully detected five bacteria: Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, and Staphylococcus aureus. The calculated sensitivity was 98%, with specificity, positive predictive value, and negative predictive value—all reaching 100%.[8]

This study demonstrates that the *MDR Direct Flow Chip* technique can identify 5 genera of species and detect 31 resistance genes. An examination was also carried out using the VITEK® 2 system, which identified 5 genus species and antibiotic sensitivity test results. The sensitivity, specificity, and comfort were obtained as 80%, 94.2%, and 94.68%. The limitation of this study is that all samples were cultured before being processed using the *MDR Direct Flow Chip* technique; none of the samples used direct specimens.

CONCLUSION

The *MDR Direct Flow Chip* technique shows potential for identifying pathogens from various specimen cultures in infected patients and could aid antimicrobial stewardship efforts in hospitals.

ETHICAL CONSIDERATIONS

This study has been approved by the local ethics committee.

FUNDING

Funding for this study was provided using a grant.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

AUTHOR CONTRIBUTION

MS and PS designed the study. RHL input and collected data and samples. MS and PS analyzed the results and wrote the first draft of the report, with revisions and input from PS. All authors contributed to revisions and approved the final version.

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