Clinical Validation of New Rapid Molecular Diagnostic Method for Bloodstream Infections

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INTRODUCTION & AIM

Bloodstream infection (BSI) is critical medical emergency associated with a high mortality rate. Rapid and accurate identification of the infectious agent and its antimicrobial susceptibility is essential for initiating the appropriate, targeted therapy and improving patient outcomes. The aim of this study was to evaluate the performance of a Molecular Mouse System (MMS) for the rapid identification of Gram-negative bacteria (GNB) and their resistance genes directly from positive blood cultures (BC).

METHOD

A total of 80 positive BC samples from different clinical departments with microscopically detected GNB were analyzed using rapid molecular multiplex assays. The Molecular Mouse system (Alifax, Polverara, Italy) is fully automated rapid PCR based test system for the qualitative detection of DNA targets by ready-to-use lab-on-chip cartridges with lyophilized reagents [1]. A positive BC is required to select the appropriate cartridge to be loaded into the system. One Molecular Mouse software session allows performing up to six simultaneous multiplex reactions.

Of the five available cartridges, two were evaluated in this study: “MM gram neg ID” cartridge for detection of the most common GNB (15 different targets) and “MM gram neg res” cartridge for selected resistance markers (13 targets). Testing was performed according to the manufacturer’s instructions. Briefly, an aliquot (200 µL) of the positive BC was centrifuged to allow plasma/bacteria separation. A second centrifugation step was performed to precipitate bacteria, which were then resuspended in DNAse free water, mixed with loading buffer and finally dispended into the cartridge wells.

Figure 1. Molecular Mouse System (two PCR devices).

CONCLUSION

MMS demonstrated good performance in the rapid detection of Gram-negative pathogens and their resistance genes directly from positive BCs. These results suggest that MMS is an effective and valuable diagnostic tool for faster therapeutic decision-making in patients with BSIs.

RESULTS

Table 1. Identification of Gram-negative BSI pathogens in Aerobic and Anaerobic Blood Cultures

Molecular Mouse result (n = 82)	
***Enterobacterales* (n = 73)**	**Non-*Enterobacterales* (n = 9)**
E. coli/Shigella spp. (n = 29)	*P. aeruginosa* (n = 4)
K. pneumoniae (n = 22)	[b]*S. maltophilia* (n = 2)
Proteus mirabilis (n = 8)	*A. baumannii* (n = 3)
Enterobacter cloacae (n = 7)	
Serratia marcescens (n = 3)	
K. oxytoca (n = 2)	
[a]*Enterobacteriacae* (n = 1)	
K. aerogenes (n = 1)	

Notes: [a]*K. aerogenes* was identified with MMS at the family level; [b]*S. maltophilia* identified with MMS in the polimicrobial sample was not detected by the routine culture. Abbreviations: AER, aerobic; ANA, anaerobic

Table 2. Identification of polymicrobial BSI pathogens

Molecular Mouse System	Routine Culture Method
E. coli/Shigella spp., *A. baumannii*	*E. coli, A. baumannii*
K. oxytoca, *P. mirabilis*, *S. maltophilia*	*K. oxytoca, P. mirabilis*
K. pneumoniae, K. aerogenes	*Enterobacter cloacae*
K. pneumoniae, E. coli	*K. pneumoniae, E. coli*

Notes: Bacteria revealed only by the MMS are underlined.

Table 3. Resistance genes detected by the Molecular Mouse system

Molecular Mouse result (n = 20)	Routine culture method
E. coli /*Shigella* spp. SHV, CTX-M-1/9 groups (n = 1)	ESBL-producing *E. coli*
E. coli /*Shigella* spp. CTX-M-1/9 groups (n = 3)	ESBL-producing *E. coli*
K. pneumoniae SHV, CTX-M-1/9 groups (n = 3)	ESBL-producing *K. pneumoniae*
K. pneumoniae SHV, CTX-M-1/9 groups, OXA-48 (n = 6)	Carbapenem-resistant *K. pneumoniae* (OXA-48 enzyme)
K. pneumoniae SHV, CTX-M-1/9 groups, NDM (n = 1)	Carbapenem-resistant *K. pneumoniae* (NDM enzyme)
K. pneumoniae SHV, CTX-M-1/9 groups, KPC (n = 1)	Carbapenem-resistant *K. pneumoniae* (KPC enzyme)
A. baumannii OXA-23 (n = 1)	Carbapenem-resistant *A. baumannii* (OXA-23 enzyme)
[a]*A. baumannii* (n = 1)	Carbapenem-resistant *A. baumannii* (unknown mechanism)
E. coli mcr-1 (n = 1)	Colistin-resistant *E. coli*
K. oxytoca CTX-M-1/9 groups (n = 1)	ESBL-producing *K. oxytoca*
P. mirabilis CTX-M-1/9 groups (n = 1)	ESBL-producing *P. mirabilis*

Notes: [a]resistance gene was not detected by the MMS.

REFERENCES

1. Molecular Mouse. Alifax. **2024**. Available online: https://www.alifax.com/products/molecular-mouse/