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Original article

Clinical evaluation of a new molecular method for the detection of multidrug-resistant microorganisms☆

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ABSTRACT

Introduction: The main objective of this work is to carry out the clinical validation of the trial with the AMR Direct Flow Chip starting from either nasal swabs, rectal swabs directly or from isolated strains to detect antibiotic resistance genes.
Methods: We developed the preclinical validation of the assay with 104 known bacterial isolates. A total of 210 nasal or rectal swab samples were analyzed. The AMR assay is based on multiplex PCR followed by reverse dot blot hybridization on DNA arrays fully automated by using the HS24 platform.
Results: Both the sensitivity and specificity of the preclinical assay were 100%, with the 104 samples correctly identified. In the clinical validation, the sensitivity was 100% and the specificity was between 100% in nasal swabs and 97% in rectal swabs.
Conclusions: The AMR Direct Flow Chip® is a rapid and effective assay for the detection of multidrug-resistant microorganisms (MDR) from nasal and rectal swab samples.

Evaluación clínica de un nuevo método molecular para la detección de microorganismos multirresistentes

Palabras clave:
Técnicas de diagnóstico molecular
Multirresistencia a fármacos en español
Análisis de microarrays
Epidemiología molecular

RESUMEN

Introducción: El objetivo fue realizar la validación clínica del sistema molecular AMR Direct Flow Chip® para la detección de genes de resistencia a antimicrobianos, partiendo de aislados bacterianos en cultivo así como de hisopos de muestras nasales o rectales.
Métodos: El ensayo AMR es una PCR multiplex seguida de hibridación reversa tipo *dot blot* en *arrays* de ADN completamente automatizada mediante la plataforma HS24 con un tiempo de realización de 3 horas. Se realizó la validación preclínica con 104 cepas bacterianas caracterizadas y posteriormente se analizaron 210 muestras de hisopos nasales o rectales.
Resultados: La sensibilidad y la especificidad del ensayo preclínico fueron del 100%, identificando correctamente las 104 cepas. En la validación clínica, la sensibilidad fue del 100% y la especificidad fue del 100% en muestras rectales y del 97% en hisopos nasales.
Conclusiones: El sistema AMR Direct Flow Chip® es un sistema rápido y eficaz para la detección de MDR a partir de muestras rectales y nasales.

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Introduction

Antimicrobial resistance is a threat to global public health,[1] since it implies an increase in morbidity and mortality.[2] Although the development of resistance is a natural biological phenomenon, the use of antibiotics has contributed to its increase.[3]

The usual time it takes to obtain the antimicrobial susceptibility of a microorganism varies between 24 and 72 h, a time that can be reduced by using rapid diagnostic tests.

There are several molecular tests for screening patients colonised by resistant microorganisms, such as the Xpert® MRSA, Xpert® vanA/vanB or Xpert® Carba-R (Cepheid Inc., Sunnyvale, CA, USA), although they still have some limitations in terms of the number of targets detected in a simple assay.[4,5]

The Antimicrobial Resistance (AMR) Direct Flow Chip® kit (Master Diagnóstica, Granada, Spain) is a molecular diagnostic system based on multiplex PCR followed by reverse dot blot hybridisation on DNA arrays, fully automated using the HS24 platform. This system makes it possible to detect 20 families of resistance genes in gram-positive and gram-negative bacteria (*mecA*, *vanA*/*B* and extended-spectrum beta-lactamase [ESBL] genes, as well as class A, B and D carbapenemase genes and the *Staphylococcus aureus* species). (Table 1). The assay can be performed directly on nasal or rectal swabs, bacterial colonies or blood cultures, with a time to result of approximately three hours.

The objective was to perform a clinical evaluation of this AMR kit for the detection of critically ill patients colonised by methicillin-resistant *S. aureus* (MRSA) in nasal swabs, or by vancomycin-resistant *Enterococcus* spp. (VRE), ESBL or carbapenemase-producing bacteria in rectal swabs.

Materials and methods

Samples

For the preclinical evaluation of the AMR kit, 104 well-characterised strains from our collection from clinical samples were used (Appendix B, Table S1). The strains were characterised using the Xpert® MRSA and Xpert® VanA/VanB (gram-positive isolates) and using a TaqMan® multiplex PCR assay on gram-negative isolates and subsequent sequencing.[6,7]

For the clinical evaluation, 210 samples (90 nasal and 120 rectal) were used, collected sequentially from patients admitted to the ICU in two independent periods of four months each. The samples were collected with transport medium swabs (Copan Diagnostics Inc., Murrieta, CA, USA) and were analysed in parallel using conventional microbiological methods and the AMR kit (using the surplus of the sample used for conventional diagnosis). The data was analysed anonymously.

Screening by conventional methods

The 90 nasal swabs for MRSA detection were cultured on ChromID® MRSA agar medium (bioMérieux, Marcy-l'Etoile, France) at 37°C for 24 h, identifying the microorganisms by MALDI-TOF. In the case of *S. aureus*, additional confirmation was performed using the Xpert® MRSA test for the detection of methicillin resistance.

For the screening of VRE, ESBL- and carbapenemase-producing strains, samples were cultured on ChromID® VRE, ESBL and Carba Smart plates (bioMérieux, Marcy-l'Etoile, France), respectively, with incubation at 37°C for 24 h. The grown microorganisms were identified by MALDI-TOF. Detection of *vanA*/*B* genes was performed using the Xpert® vanA/vanB system, and that of ESBL-, carbapenemase- and AmpC-encoding genes, using previously published methods.[8]

Assay with the AMR Direct Flow Chip® kit

For preclinical validation, strains were seeded on blood agar medium (5% Columbia Blood Agar; bioMérieux, Marcy-l'Etoile, France) and incubated at 37°C, for 18–24 h. After resuspending a single colony in 100 μl of sterile distilled water, 5 μl of that sample were taken and made up to 50 μl with the kit's amplification reagents (43.5 μl of buffer solution and 1.5 μl of the PCR enzyme).

For clinical validation, nasal and rectal swabs were resuspended in 0.5 ml of sterile water and mixed by shaking. For the PCR, 5 μl of the nasal swab suspension and 5 μl of a dilution (1/10) of the rectal swab suspension were used. The PCR was performed as previously described (GeneAmp® PCR System 9600 thermocycler; Thermo Fisher Scientific, Waltham, MA, USA), as follows: 10 min at 25 °C; 3 min at 95 °C; 40 cycles at 95 °C for 15 s, followed by 45 s at 50 °C, 1 min at 72 °C and storage at 4 °C. The total time was 2 h.

After performing the PCR, the hybriSpot HS24 automated platform (Master Diagnóstica, Granada, Spain) was used (Fig. 1A), which performs a reverse dot blot hybridisation of up to 24 samples simultaneously and interprets the results in one hour. When the specific amplicons hybridise with their corresponding probes, the signals are visualised through a colorimetric reaction (Fig. 1B and C). The hybriSpot HS24 platform captures the image of the chip, which is automatically analysed using a pattern of points that corresponds to a profile of genetic determinants of resistance (Fig. 1).

Statistical analysis

Contingency tables were calculated taking into account the results obtained by conventional techniques (gold standard) and by the AMR kit. Sensitivity, specificity and 95% confidence intervals were calculated using the SPSS v17.0 software.

Results and discussion

Preclinical validation results

The results obtained in the preclinical validation with the AMR kit obtained 100% concordance with the expected results for the collection of strains used (see supplementary material, Appendix B, Table S1).

Table 1
Genetic determinants of resistance detected by the AMR Direct Flow Chip® assay.

Microorganisms			Genetic determinants of resistance
Gram-positive bacteria	Methicillin-resistant *Staphylococcus aureus*	–	SA-*mec*
	Vancomycin-resistant *Enterococcus* spp.	–	*vanA and vanB*
Gram-negative bacteria ESBL and carbapenemase producers	*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, *Acinetobacter baumannii*, *Serratia marcescens*, *Enterobacter asburiae*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*	Class A	$bla_{\text{CTX-M}}$, bla_{SHV}, bla_{SME}, bla_{KPC}, $bla_{\text{NMC/IMI}}$ and bla_{GES}
		Class B	bla_{IMP}, bla_{GIM}, bla_{VIM}, bla_{SPM}, bla_{SIM} and bla_{NDM}
		Class D	$bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-48-like}}$, $bla_{\text{OXA51-like}}$ and $bla_{\text{OXA-58-like}}$

ESBL: extended spectrum beta-lactamase.

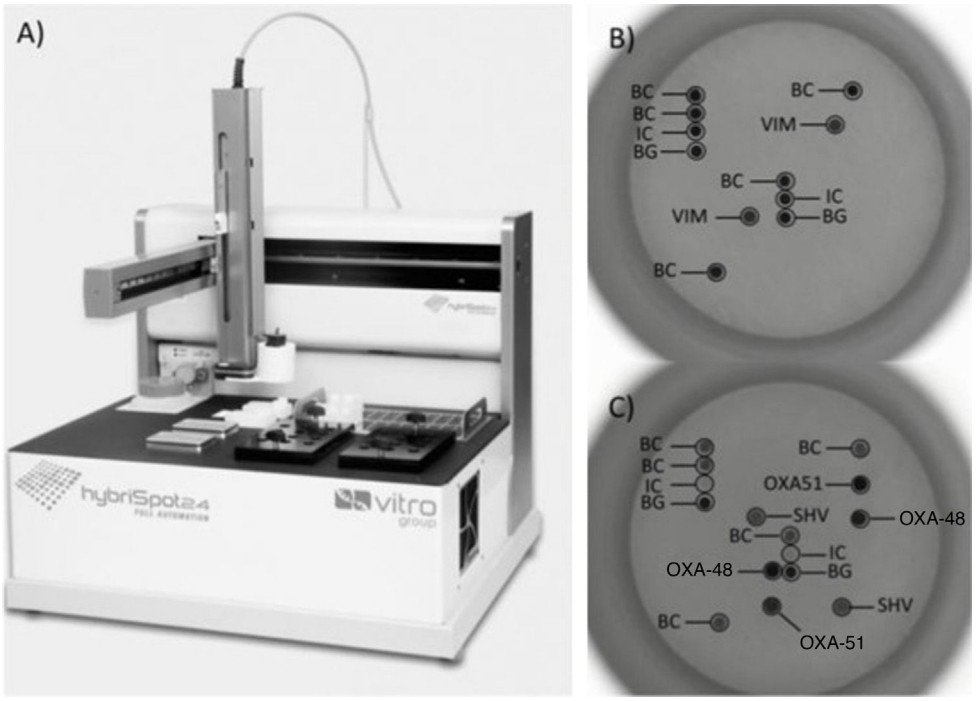

Figure 1. HS24 platform and AMR Direct Flow Chip®. A) HS24 automated platform for reverse hybridisation processing and image analysis. B) Results for a carbapenemase-producing *Escherichia coli* strain. C) Detection of MDR organisms in a positive rectal swab harbouring *Klebsiella pneumoniae* bla_{OXA-48}/bla_{SHV} and *Acinetobacter baumannii* bla_{OXA-51}. All the probes are spotted in duplicate on the chip array and a result is considered positive if both signals are detected. Positive probes detected: biotin control (BC); exogenous amplification control (IC), for the detection of synthetic DNA included in the PCR mix; endogenous amplification control (BG), for the detection of the human *beta-globin* gene, bla_{VIM} (VIM), bla_{SHV} (SHV), bla_{OXA-48} (OXA48) and bla_{OXA-51} (OXA51). The image is magnified ×5 compared to the actual size of the chip.

Table 2
Sensitivity and specificity for the assays performed using the AMR Direct Flow Chip® on nasal and rectal swabs for the detection of genetic determinants of resistance.

Resistant microorganisms		Number of positive samples	Number of samples detected by the AMR kit	AMR result	Sensitivity (%)	95% CI	Specificity (%)	95% CI
Nasal swabs (n = 90)								
MRSA		8	10	*S. aureus* + mecA	100	59–100	97	90–99
Rectal swabs (n = 120)								
ESBL								
K. pneumoniae[12], *E. coli*[10], *A. baumannii*[1]	bla_{CTX}	23	23	bla_{CTX}	100	82–100	100	95–100
K. pneumoniae[5]	bla_{SHV}	5	5	bla_{SHV}	100	46–100	100	96–100
K. oxytoca[1]	bla_{GES}	1	1	bla_{GES}	100	5–100	100	96–100
Carbapenemase producers		9	9	–	100	62–100	100	96–100
K. pneumoniae[6], *E. coli*[2]	bla_{VIM}	8	8	bla_{VIM}	100	60–100	100	95–100
A. baumannii[1]	bla_{OXA-23}/bla_{OXA-51}	1	1	bla_{OXA-23} + bla_{OXA-51}	100	5–100	100	96–100
VRE	6	6	–	100	52–100	100	96–100	
E. faecium[2]	*vanA*	2	2	*vanA*	100	20–100	100	96–100
E. faecium[3], *E. faecalis*[1]	*vanB*	4	4	*vanB*	100	40–100	100	96–100

AMR: AMR Direct Flow Chip®; ESBL: extended spectrum beta-lactamase; VRE: vancomycin-resistant *Enterococcus*; 95% CI: 95% confidence interval; MRSA: methicillin-resistant *Staphylococcus aureus*.

Clinical validation results

After analysing 90 nasal swabs in parallel for MRSA detection, the conventional method detected 8 positive and 82 negative samples, while the AMR kit detected 10 positive (*mecA*-positive gene) and 80 negative samples, with two false positives. The sensitivity and specificity in clinical samples were 100% and 97%, respectively (Table 2).

Regarding rectal swabs, 30 positive samples for beta-lactamase production were detected using conventional methods, of which 24 were classified as ESBL and six as *ampC* producers. The results obtained with the AMR kit are shown in Table 2. Among the 24 samples in which ESBL was detected, the AMR kit simultaneously detected swabs containing microorganisms with more than one genetic marker of resistance, one sample with a strain of *Acinetobacter baumannii* with the bla_{CTX}/bla_{OXA-51} genes and five samples with *Klebsiella pneumoniae*, a producer of ESBL and carbapenemases (bla_{CTX}/bla_{SHV}/bla_{VIM}). The presence of *ampC* genes was detected in six samples, three of them with *Escherichia coli* with bla_{CIT}, one with *A. baumannii* with bla_{MOX} and two with *Enterobacter cloacae* with the bla_{EBC} chromosomal gene. For the detection of carbapenemase-producing strains, the conventional method indicated nine positive samples, in which the microorganisms carrying these genetic markers of resistance were *K. pneumoniae*, *E. coli* and *A. baumannii*. Regarding the detection of VRE, the AMR kit showed absolute concordance with the results obtained by conventional methods. Five of the six positive samples contained *Enterococcus faecium* and the remaining one contained *Enterococcus faecalis* with *vanA* or *vanB* genes. These results are shown in Table 2. The sensitivity and specificity of the kit were 100% on rectal swabs.

The main advantages of this test were the time to result (three hours) and the variety of genetic determinants of resistance that it detects. However, two false positives for MRSA were obtained on nasal swabs. This limitation could be explained by the technology used in the kit, since it independently detects the presence of *S. aureus* genes and the *mecA* gene. In the case of gram-negatives, the limitation was the non-identification of *ampC*-carrying microorganisms, which could be a problem for implementing infection control policies.

According to the results of this preliminary study, the AMR kit is a versatile test for the screening of patients with multiresistant microorganisms directly from clinical samples, either through nasal or rectal swabs, reproducing results similar to those obtained by other diagnostic tests, such as the Xpert® MRSA[9] or Xpert® Carba-R.[10]

Using a simple and direct assay, the method detects 21 targets, including MRSA, VRE, ESBL and carbapenemases, so it can be a useful tool in resistance surveillance programmes. However, additional studies should be carried out to analyse the long-term clinical impact of this new diagnostic tool.

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Conflicts of interest

To carry out this study, the company Master Diagnóstica provided the research team with the AMR Direct Flow Chip® diagnostic kits.

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Some of the data were obtained from the usual diagnostic work carried out in the Microbiology Service of the General University Hospital of Elche.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eimce.2022.04.005.

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