A multicentric validation of a rapid detection test for MCR-1 producing bacteria

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Abstract
The emergence and spread of Enterobacteriaceae resistant to critically important antibiotics such as carbapenems is a matter of great public Health concern. Accordingly, colistin, a cationic polypeptide introduced in the 1950s, has become a last-resort molecule that has been increasingly prescribed in clinics in the last years. Of note, colistin is also widely used in the food animal sector. Late 2015, a plasmid-born colistin resistant gene (mcr-1) was reported, and a rapid detection of MCR-1-producing bacteria is essential to prevent further spread and provide appropriate antimicrobial therapy. Here, we provide validation data of a Lateral Flow Immunoassay (LFIA) to detect MCR-1 producers within 15 minutes.

Background
MICROBIAL ANTIRESISTANCE (mAbs), PRODUCTION AND SELECTION
Mice were immunized with purified recombinant periplasmic region of MCR-1 (p-MCR-1) 20 mAbs were selected and purified

Methods
LFIAs (strip + cassette) were manufactured using monoclonal antibodies previously produced and selected. A retrospective collection of mcr-1-positive enterobacterial isolates of human and animal origin was tested in a multicentric way. The isolates were grown on agar and one colony was overlayed in extraction buffer and then dispersed on the cassette. Migration was allowed for 15 minutes and results were monitored by the appearance of a specific band.

Results
Positive results showed a dark pink colored band leading to no ambiguous interpretation. All mcr-2-positive isolates were detected as positive by the LFIA and no false negative result was observed. Three out of four strains producing MCR-2 were detected positives. Our test does not detect MCR-3; MCR-4 or MCR-5 producing bacteria.

Conclusions
Our LFIA is able to detect MCR-1 with 100% sensitivity and 98% specificity. It also appears usable for the detection of MCR-2 (3/4) but further studies are needed to better assess the performances of MCR-2 detection. It is compatible with samples handled in laboratories. This test is rapid, sensitive, specific, easy to use, cost-effective and could thus be implemented in any microbiology laboratory around the world. Considering the relevance of colistin resistance in humans and animals, and the possible transfers of MCR producers between them, the LFIA test could be of major help for diagnostic and monitoring purposes in the two sectors.

1.4. coli MCR-1
2. C. freundii CTX-M-1
3. Enterobacter cloaceae
4. K. oxytoca CTX-M-14
5. K. pneumoniae CTX-M-15
6. E. coli CTX-M-18
7. E. cloacae
8. S. marcescens RMP-11
9. C. jejuni (DIA-48 260)
10. E. faecium (DIA-38 122)
11. K. oxytoca OXA-183
12. E. coli OXA-244

IMMUNOCHEMOTRANSFER ASSAY PRINCIPLE

Results

Limit of detection (migration time 15 minutes)

Detection

Positive test is obtained only with the strain producing MCR-1 all other tests are negatives whatever the strain or beta-lactamase produced

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Conclusions and perspectives

Our LFIA is able to detect MCR-1 with 100% sensitivity and 98% specificity
Our test might be implemented in any human or animal or microbiology diagnostic laboratory
Our LFIA is easy to use, rapid, sensitive
The Ng-Test MCR-1 is commercially-available
The Ng-Test MCR-1 could be a very useful tool for the monitoring of the MCR-1 dissemination

1 MCR-1 producing colistin resistant
3 MCR-3 producing colistin resistant
5 MCR-5 producing colistin resistant
7 no MCR producing strains colistin susceptible
9 no MCR producing strains colistin susceptible

MCR-1 lateral flow immunoassay (LFIA) development

NG-Test MCR-1 validation: manufactured tests from BioTech

Retrospective validation
- 298 strains from 3 different locations: Hôpital Bicêtre (Paris, France); Anses (Lyon, France) and CHU Clermont-Ferrand (Clermont Ferrand, France)
- Origin: Isolates from human (177) or animal (121) samples
- Isolates: 221 Escherichia coli; 48 Klebsiella pneumoniae; 16 Enterobacter cloacae; 8 Salmonella sp; 2 Hafnia alvei; 1 Shewanella oneidensis; 1 Shewanella profunda; 1 Citrobacter freundii
- 109 MCR-1 producing strains and colistin resistant
- 1 MCR-1 producing strains and colistin susceptible (MIC<0.5 µg/ml)
- 4 MCR-2 producing strains and colistin resistant
- 16 MCR-3 producing strains and colistin resistant
- 2 MCR-3 producing strains and colistin susceptible (MIC<0.5 µg/ml)
- 5 MCR-4 producing strains and colistin resistant
- 3 MCR-5 producing strains and colistin resistant
- 95 non MCR producing strains and colistin susceptible
- 67 non MCR producing strains and colistin susceptible (MIC<0.5 µg/ml)

Protocol
One colony from agar plate was suspended in 150µl of extraction buffer (extraction step). After vortexing 100µl were loaded on the cassette. Migration 15' before naked eye reading.

The tests were realized at the 3 locations.

Results
All the MCR-1 producing strains gave a positive result, except 1 MCR-1 producing but colistin susceptible isolate 3/4 of the MCR-2 producing isolates gave a positive test result
All the other strips gave a negative result

Some test examples

- MCR-1 producing colistin resistant
- MCR-3 producing colistin resistant
- MCR-5 producing colistin resistant
- No MCR producing strains colistin resistant
- No MCR producing strains colistin susceptible

Conclusion

The emergence and spread of Enterobacteriaceae resistant to critically important antibiotics such as carbapenems is a matter of great public Health concern. Accordingly, colistin, a cationic polypeptide introduced in the 1950s, has become a last-resort molecule that has been increasingly prescribed in clinics in the last years. Of note, colistin is also widely used in the food animal sector. Late 2015, a plasmid-born colistin resistant gene (mcr-1) was reported, and a rapid detection of MCR-1-producing bacteria is essential to prevent further spread and provide appropriate antimicrobial therapy. Here, we provide validation data of a Lateral Flow Immunoassay (LFIA) to detect MCR-1 producers within 15 minutes. The LFIA is able to detect MCR-1 with 100% sensitivity and 98% specificity. It also appears usable for the detection of MCR-2 (3/4) but further studies are needed to better assess the performances of MCR-2 detection. It is compatible with samples handled in laboratories. This test is rapid, sensitive, specific, easy to use, cost-effective and could thus be implemented in any microbiology laboratory around the world. Considering the relevance of colistin resistance in humans and animals, and the possible transfers of MCR producers between them, the LFIA test could be of major help for diagnostic and monitoring purposes in the two sectors.