

Development of a rapid diagnostic test for identification of group 1 and group 9 CTX-M β -Lactamases

Q. Gillemann¹, S. Debière¹, F. Nazé¹, L. Avrain¹, Y. Nizet², J. Bouzet¹, L. Denorme¹, P. Mertens¹.

¹Coris BioConcept - Gembloux (Belgium), ²SynAbs - Gosselies (Belgium)

Introduction

Amongst β -lactamases, those with an extended spectrum (ESBL) confer resistance to most beta-lactams, reducing the therapeutic window for the treatment of bacterial infections. The CTX-M family, mainly represented by CTX-M-15 (group 1) and CTX-M-14 (group 9), is an important part of these ESBLs.

RESIST CTX-M test for identification of CTX-M types 14 and 15 is a tool allowing a more rational use of last resort antibiotics and the monitoring of the epidemiological situation.

Methods

The development of this test is based on capture antibodies specifically produced in the aim of encountering 2 requirements:

- specificity (distinction) to each target (CTX-M-15 and CTX-M-14);
- ability to capture more widely the variants inside the groups 1 and 9.

Step 1: Immunization with whole sequence proteins or with peptides bioinformatically designed to be accessible by the antibodies based on 3D structure of the proteins (Figure 1) and highly immunogenic (based on 2D motifs).

Step 2: Selection of monoclonal antibodies reacting with the whole target proteins, ensuring the recognition of the native enzymes. Detection gold-labelled antibody is selected from the group of antibodies recognizing both CTX-M groups 1 and 9 enzymes.

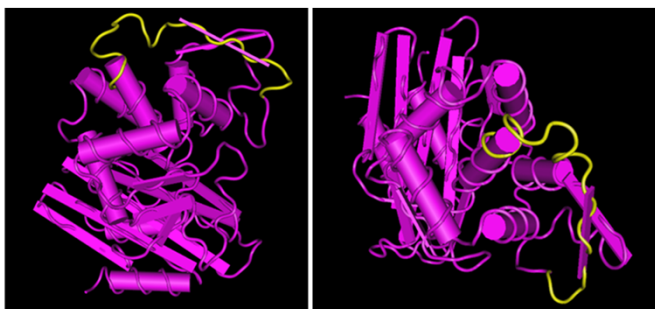


Figure 1 : Representation of highly immunogenic target peptide (yellow) in the 3D structure of CTX-M-15 protein (Group 1).

Results

The 136 candidate monoclonal antibodies were grouped in 3 categories:

- recognition of CTX-M-15 (group 1) alone;
- recognition of CTX-M-14 (group 9) alone;
- recognition of both CTX-M-15 and CTX-M-14 (Figure 2).

These antibodies were all tested, looking for the best combination allowing the development of an immunochromatographic test design able to detect and distinguish group 1 and group 9 CTX-M (two test lines).

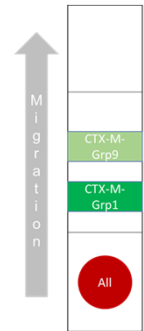


Figure 2 : The test lines capture specifically the CTX-M group 1 or group 9. The gold-conjugated detection antibody can reveal CTX-M's captured by any of the test lines.

Bacterial strains (4 *Klebsiella pneumoniae* and 15 *Escherichia coli*), all producing a CTX-M enzyme (11 from group 1 CTX-M including CTX-M-1-3-15 and 8 from group 9 CTX-M including CTX-M-9-14-24-27) were tested. All bacterial strains were properly identified as either group 1 or group 9 CTX-M producer, following their status (Figure 3).

Strain	Variant	Group	Test result
<i>E.coli</i>	CTX-M-9	9	Grp9
<i>E.coli</i>	CTX-M-14	9	Grp9
<i>E.coli</i>	CTX-M-24	9	Grp9
<i>E.coli</i>	CTX-M-27	9	Grp9
<i>E.coli</i>	CTX-M-9	9	Grp9
<i>E.coli</i>	CTX-M-14	9	Grp9
<i>E.coli</i>	CTX-M-27	9	Grp9
<i>K.pneumoniae</i>	CTX-M-14	9	Grp9
<i>E.coli</i>	CTX-M-1	1	Grp1
<i>E.coli</i>	CTX-M-3	1	Grp1
<i>E.coli</i>	CTX-M-15	1	Grp1
<i>K.pneumoniae</i>	CTX-M-15	1	Grp1
<i>K.pneumoniae</i>	CTX-M G1	1	Grp1
<i>K.pneumoniae</i>	CTX-M G1	1	Grp1
<i>E.coli</i>	CTX-M-15	1	Grp1
<i>E.coli</i>	CTX-M-1	1	Grp1
<i>E.coli</i>	CTX-M-1	1	Grp1
<i>E.coli</i>	CTX-M-3	1	Grp1
<i>E.coli</i>	CTX-M-15	1	Grp1

Figure 3 : Results of the 19 strains tested on RESIST CTX-M test.

In order to validate the RESIST CTX-M test, external clinical evaluations have been conducted and confirmed the good performances. For more details, see the Coris website (QR code).



Conclusion

The peptidic/proteic based strategy implemented by **Coris BioConcept** succeeded in orienting the production of highly specific antibodies which were used to develop the **RESIST CTX-M test** able to detect and identify group 1 and group 9 CTX-M ESBL producing bacteria.

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