Haut Conseil de la santé publique -
French High Council for Public Health

# OPINION

## regarding the microbiological diagnosis of carbapenemase-producing *Enterobacteriaceae* or colistin-resistant *Enterobacteriaceae* carrying the *mcr-1* gene

6 December 2016

On 4 August 2016, the High Council for Public Health (HCPH) received a referral from the General Directorate of Health (GDH) regarding measures to be taken with regard to the emergence of a plasmid-mediated (*mcr-1*) colistin resistance in *Enterobacteriaceae*.

This referral to the HCPH from the GDH arises following the recent notification in the United States of two cases of patients carrying strains of *Escherichia coli* with the *mcr-1* gene, likely to give rise to a colistin resistance, and the reporting of 25 July 2016 by the Institut Pasteur laboratory in Noumea (New Caledonia) of the first detection in France of a strain of *Enterobacteriaceae* carrying the *mcr-1* gene, also producing an extended spectrum beta-lactamase (ESBL). Plasmid-mediated colistin resistance has also been identified since then in collections of strains of Enterobacteriaceae in Spain and in France.

This referral to the HCPH from the GDH was followed by the issue of a Quick Health Alert Message (QHAM) to health establishments, dated 2 September 2016 [1].

To reply to the first question raised by the referral of the GDH (updating the definition of the emerging highly-resistant bacteria eHRB), the HCPH issued a first report on 27 September 2016 regarding the measures to be taken by health establishments with regard to plasmid-mediated (*mcr-1*) colistin resistance in *Enterobacteriaceae* [2].

The aim of this report is to reply to the second question of the referral regarding means of identifying colistin resistant strains of *Enterobacteriaceae* by the presence of the *mcr-1* gene. Upon the occasion of the issue of this opinion, the HCPH is updating the chapter “Screening for and microbiological diagnosis of eHRB recommendations for the prevention of cross-transmission of “Highly-Resistant Emergent Bacteria (eHRB)” published in 2013 [3].

**The HCPH points out the following**:

- *Enterobacteriaceae*’s acquired colistin resistance is mainly due to an enzymatic modification of the lipopolysaccharide (LPS), which tends to reduce intracellular penetration of the antibiotic [4]. The lipid A phosphate groups can be substituted by molecules of 4-amino-4-deoxy-L-arabinose or of phosphoethanolamine [5-7]. These modifications are catalysed by enzymes conserved within species of bacteria, whose expression is increased following the alteration of regulator genes. The decrease in the net load of LPS which reduces the affinity of colistin for LPS and leads to resistance. The overexpression of outflow systems and a capsular trap of the colistin may also confer a resistance [8,9].

- Until very recently, acquired colistin resistance was uniquely attributed to the presence of chromosome mutations in clinical human isolates. However, on 18 November 2015, the first plasmid-mediated colistin resistance mechanism was described in China in animals, human or food *Enterobacteriaceae* [10]. The corresponding gene, *mcr-1*, codes for a plasmid phosphoethanolamine transferase, that confers a low level of resistance to colistin (modal minimum inhibitory concentration [MIC]: 4 mg/L). Its prevalence in China has been estimated at approximately 20% in animals and 1% in humans.
- A close variant, *mcr-1/2*, sharing 99% of the identity of *mcr-1* has been described, as well as a more distant variant, mcr-2 (76.7% identical to *mcr-1*), thus far reported solely in animals (Belgium). The *E. coli* species represents more than 80% of the *mcr-1* positive isolates, but the genus *Klebsiella*, *Salmonella*, and more rarely *Shigella* and *Enterobacter* can also propagate the gene. Various samplings of bacterial collections studied to date mean that it is possible to conclude that the gene has been detected, but not yet at levels of prevalence which are validated on an epidemiological level.
- The first *mcr-1* gene detected in China was carried by a plasmid of the incompatibility group IncI2 presenting this sole resistance gene. Since then, the gene has been detected on plasmids belonging to incompatibility groups IncHI2, IncHI2A, IncX4 and IncP [11-14]. The *mcr-1* gene has been observed frequently in bacteria producing an ESBL of the cefotaximase-Munich (ESBL CTX-M), more rarely in NDM carbapenemase-producing isolates (for New Delhi metallo-beta-lactamase), OXA-48 and KPC (for *Klebsiella pneumoniae* carbapenemase) [15-18]. The co-location of *mcr-1* and of genes coding for a CTX-M-type ESBL on the same plasmid has been reported in different geographical areas including Europe. The plasmid colocation of *mcr-1* with the gene coding for the NDM-5 carbapenemase has been observed in China [19]. In France, only a few strains of *E. coli mcr-1* have been characterised to date in human medicine. In 2016, five were isolated in metropolitan France, two of which produced carbapenemases; NDM on the one hand, OXA-48 plus KPC on the other hand (data from the National Reference Centre - CNR).
- There is currently no commercially available selective culture medium for detecting colistin-resistant *Enterobacteriaceae* from clinical samples. The composition of a selective agar culture medium has nonetheless been published [20]. It has not yet undergone independent assessment. It enables all colistin-resistant *Enterobacteriaceae* to be detected including isolates without ESBL or carbapenemase.
- The characterisation of suspect strains includes the determination of the MIC of colistin by micro-dilution in a liquid medium. Resistance is most often expressed at a low level with reported MIC values of between 2 and 16 mg/L and median MIC of 4 mg/L, which corresponds to the limit value defining resistance according to the critical concentrations of the CA-SFM/EUCAST [21].
- There is currently little hindsight on the screening performance of these *Enterobacteriaceae* using automated testing methods of antibiotic susceptibility in liquid media.

As current epidemiological knowledge stands, the HCPH recommends the following measures for detecting colistin resistance associated with the *mcr-1* gene:

- To determine the MIC of colistin by the reference method of microdilution in liquid medium, according to the recommendations of the CA-SFM/EUCAST [21].
- To use:
    - A Mueller-Hinton liquid medium with an adjustment of the concentration in cations (MH2),

  - Colistin sulfate salt (methanesulfonate used in therapy is a prodrug of colistin, inactive in vitro),
  - Untreated polystyrene microplates or strips.
- Not using additives such as polysorbates.
- To attribute a detected resistance by measuring the MIC to the expression of a *mcr* gene, detect the presence of the gene using molecular techniques, which are the only ones enabling a specific diagnosis with certainty.
  - The CNR can make characterised strains available to be used as control strains in the event of laboratories applying molecular biology techniques.
  - In any event, suspect strains must be sent to the CNR of resistance to antibiotics (Clermont-Ferrand center) for confirmation and surveillance of the epidemiological situation on a national scale. It should be noted that ESBL or carbapenemase producing strains sent to the CNR are systematically screened for resistance to colistin and for the presence of *mcr-1/2* genes.

**In revising the 2013 recommendations for the microbiological screening and detection of carbapenemases (CPE) producing Enterobacteriaceae, the HCPH proposes the following formulation for chapter "2-7-1: Principles in testing for gut carried eHRB", paragraph "Testing for CPE" (pages 32 and 33 of the 2013 report) [3]:**

- Systems testing specifically for CPE in stools using molecular biology are now available commercially.
- Failing these systems, it is recommended that any pathology laboratory in charge of health establishments have available at all times two types of agar on which an aliquot of stools or a suspension from a rectal swab can be streaked out:
  - agar enabling 3rd generation (C3G) cephalosporin-resistant strains to be grown, including those producing Amber A or B class carbapenemase,
  - and agar specifically intended for growing Ambler class D carbapenemase-producing strains.
- An identification of the colonies growing on these media and standard antibiotic susceptibility testing (agar diffusion test) according to CA-SFM/EUCAST are carried out [21].
- If the antibiotic susceptibility test shows non-sensitivity to one or more of the carbapenems tested for other than ertapenem (ERT), the strain can be declared as non-sensitive to carbapenems. However, if the non-sensitivity only concerns the ERT (inhibition zone diameter < 25 mm or according to the automatic analyser: "estimated" MIC ≥ 0,5 mg/L), the strain can only be declared non-sensitive to ERT after confirmation that the ERT's MIC is > 0,5 mg/L.
- The following antibiotics must be tested with standard antibiotic susceptibility testing (ticarcillin + clavulanic acid, temocillin, imipenem or meropenem and cefepime) as they are used in the algorithm to screen CPEs within Enterobacteriaceae that are non-sensitive to carbapenems [21].
- A laboratory that detects a suspect strain according to the result of the carbapenemase screening algorithm and that does not have the tools to prove it, contacts the competent laboratory with which it forges functional ties, or with the CNR of resistance to antibiotics, to determine within a short time (4 days maximum, delivery time included) whether or not the strain produces a carbapenemase.

- There are commercially available tests to detect the production of carbapenemase using enzymological and immunochromatographic methods.

**These recommendations have been drawn up on the basis of knowledge available at the date of publication and are subject to modification in the event of new data being available.**

Opinion drawn up by a group of experts, members or non-members of the HCPH, around the Expert Committees “Patient Safety: Nosocomial infections and other undesirable events linked to treatments and practices” and “Infectious Diseases”. No conflict of interest has been identified.

The expert committee on “Patient Safety: Nosocomial infections and other undesirable events linked to treatments and practices” met on 6 December 2016; 10 qualified members out of 15 qualified voting members were present, 1 conflict of interest was identified: the text was approved by 9 votes, 0 vote against, 0 abstention.

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Opinion produced by the Expert Committee on Patient safety: nosocomial infections and other undesirable events linked to treatment and practices

6 December 2016

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