

## NIVB Meeting 2022

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The first annual meeting of the National Institute of Virology and Bacteriology (NIVB)

## Organizers:

Institute of Organic Chemistry and Biochemistry of the CAS

Masaryk University

Charles University

University of Chemistry and Technology Prague

Palacký University Olomouc

Institute of Molecular Genetics

of the CAS

Institute of Microbiology of the CAS

**Biology Centre CAS** 

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## L-24 PREFERRED β-LACTONE SYNTHESIS CAN EXPLAIN HIGH RATE OF FALSE-NEGATIVE RESULTS IN THE DETECTION OF OXA-48-LIKE CARBAPENEMASES

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The resistance to carbapenems is usually mediated by enzymes hydrolyzing β-lactam ring. Recently, an alternative way of the modification of the antibiotic, a β-lactone formation by OXA-48-like enzymes, in some carbapenems was identified. We focused our study on a deep analysis of OXA-48-like-producing Enterobacterales, especially strains showing poor hydrolytic activity. In this study, well characterized 74 isolates of Enterobacterales resistant to carbapenems were used. Carbapenemase activity was determined by matrix-assisted laser desorption/Ionization time-of-flight mass spectrometry (MALDI-TOF MS), liquid chromatography/mass spectrometry (LC-MS), and Carba-NP test. As meropenem-derived  $\beta$ -lactone possesses the same molecular weight as native meropenem (MW 383.46 g/mol), β-lactonization cannot be directly detected by MALDI-TOF MS. In the spectra, however, the peaks of m/z=340.5 and 362.5 representing decarboxylated β-lactone and its sodium adduct were detected in 25 out of 40 OXA-48-like producers. In the rest 15 isolates, decarboxylated hydrolytic product (m/ z=358.5) and its sodium adduct (m/z=380.5) have been detected. The peak of m/z=362.5 was detected in 3 strains co-producing OXA-48-like and NDM-1 carbapenemases. The respective signal was identified in no strain producing class A or class B carbapenemase alone showing its specificity for OXA-48-like carbapenemases. Using LC-MS, we were able to identify meropenem-derived β-lactone directly according to the different retention time. All strains with a predominant βlactone production showed negative results of Carba NP test. In this study, we have demonstrated that the strains producing OXA-48-like carbapenemases showing false-negative results using Carba NP test and MALDI-TOF MS preferentially produced meropenem-derived β-lactone. We also identified  $\beta\mbox{-lactone-specific}$  peak in MALDI-TOF MS spectra and demonstrated the ability of LC-MS to detect meropenemderived \beta-lactone.

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