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### **Colistin resistance among carbapenem-resistant Enterobacteriaceae recovered in Belgium in 2014-2015**

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**Background:** *mcr-1* plasmid-mediated resistance to colistin was reported for the first time in 2015 raising the concern of its occurrence in multidrug or in extensively-resistant Enterobacteriaceae. Therefore we aimed to assess the frequency of colistin resistance and the associated mechanisms of resistance among carbapenem-resistant *Klebsiella pneumoniae* (KP) and *Escherichia coli* (EC) isolates referred for confirmation of carbapenemase-producing Enterobacteriaceae to the national reference centre in 2014 and in 2015.

**Material/methods:** All non-duplicate, clinical KP and EC isolates with decreased susceptibility to carbapenems (EUCAST or CLSI criteria) sent for confirmation between 1/2014 and 12/2015 were tested for MIC susceptibility to colistin and to carbapenems (meropenem, ertapenem and imipenem) by broth microdilution using Sensititre panels (Thermo Fisher, UK). Carbapenemase encoding genes, *mcr-1* plasmidic gene and the *mgrB* gene involved in chromosomal resistance to colistin were sought by PCR/sequencing.

**Results:** Among carbapenem non-susceptible KP (n=791) and EC (n=152) isolates, 101 KP (12.7%) and 2 EC (1.3%) were resistant to colistin (CR-KP & CR-EC) (MICs from 4 to  $\geq 16$  mg/L). Of the 74 KP isolates available and analyzed for colistin resistance mechanisms, 56 CR-KP harbored a carbapenemase encoding gene (OXA-48-like [n=30], KPC [n=18], OXA-427 [n=4], NDM, NDM+OXA-48-like, VIM [n=1, each]) and 18 were non-carbapenemase producing isolates. No *mcr-1* gene was found in any of the CR-KP but 44 isolates (59%) presented mutations in the chromosomal negative regulator *mgrB* gene (insertion sequence (IS) [n=19], amino acid substitutions (n=4), non sense

mutations (n=8), indels (n=4), deletion/transposition of the entire *mgrB* gene (n=9)). The two CR-EC isolates harboured *bla*<sub>OXA-48</sub> and *mcr-1* gene (MIC to colistin = 4 mg/L). The rate of colistin resistance among carbapenem-non-susceptible KP and EC isolates increased significantly from 8% (32/411) in 2014 to 14% (70/517) in 2015 (p=0.005) mostly because of the doubling colistin resistance rate among KPC-producing KP between 2014 (14%; 10/72) and 2015 (33%; 29/87).

**Conclusions:** In 2014 and 2015, colistin resistance was found in respectively 13% and 1% of the carbapenem non-susceptible KP and EC received for carbapenemase suspicion. The high rate of colistin resistance observed in KP could be related to the expansion of specific KPC-producing KP clone in Belgium. *mcr-1* gene was found in Belgium in two OXA-48-producing *E. coli*. In carbapenem resistant KP, colistin resistance was mostly related to abnormalities in the chromosomal *mgrB* genes and not to the presence of *mcr-1* plasmids. The increase of colistin resistance among carbapenem-resistant and multidrug resistant KP and EC isolates raises major concern for therapeutic management of multidrug resistant infections and underlines the need for careful continuous epidemiological monitoring.