27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P031 The mcr genes around the world

Category: 3b. Resistance surveillance & epidemiology: Gram-negatives

23 April 2017, 12:30 - 13:30
P0687

Resistance to colistin and emergence of the mcr-1 gene among Enterobacteriaceae in a general hospital in Spain

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Background: Increasing antibiotic resistance in Enterobacteriaceae and the emergence of carbapenemase-producing isolates has renewed the interest in the use of polymyxins for treating multidrug resistant infections. Although nowadays resistance to colistin among Enterobacteriaceae is infrequent and, in general, non-transferable, the recent description of transferable colistin resistance due to the *mcr-1* gene mainly in animal isolates is a cause of concern. The aim of this study was to determine the prevalence of resistance to colistin among Enterobacteriaceae isolated in our laboratory and to analyze the resistant isolates for the presence of the *mcr-1* gene.
Material/methods: Over a period of 1 year (August 2015-July 2016) we prospectively determined the activity of colistin against all Enterobacteriaceae isolated in our laboratory from clinical samples. Susceptibility testing was performed by using the microdilution MicroScan® Neg MIC 44 panel. All isolates showing resistance to colistin (MIC >2 mg/L; EUCAST breakpoint), excluding the intrinsically resistant species, were also tested by the Etest method (bioMérieux, France). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control strains. In addition, the isolates were screened for the presence of the *mcr-1* gene by PCR with the primers CLR5-F (5'-GGTCAGTCCGTTTGTTC-3') and CLR5-R (5'-CTTGGTCGGTCTGTA GGG-3') as described by Liu et al. (Lancet Infect Dis. 2015).
Results: A total of 4,394 Enterobacteriaceae were analyzed. Among them, 108 isolates (2.4%) were colistin resistant (MIC >2 mg/L). The isolates corresponded to *Enterobacter cloacae* complex (n=76), *Klebsiella pneumoniae* (n=24), *E. coli* (n=5), and *Salmonella enterica* (n=3). The source of the

isolates was wound/abscess (n=46), lower respiratory tract (n=29), blood (n=13), peritoneal fluid (n=10), rectal swabs (n=5), feces (n=3), and others (n=2). Colistin MIC50, MIC90, and range for all resistant isolates tested were 64, >256, and 4->256 mg/L, respectively. All *E. cloacae* complex isolates showed hetero-resistance to colistin.
The presence of the *mcr-1* gene was only detected in one *E. coli* isolate also producing the extended-spectrum beta-lactamase CTX-M-15. The MIC of colistin against the isolate was 4 mg/L (both by microdilution and by Etest). The source of the isolate was a rectal swab from a patient with cancer that was not previously treated with colistin.
Conclusions: Although to date the rate of colistin resistance among clinical isolates of Enterobacteriaceae in our institution is not high (2.4%), the emergence of *E. coli* carrying transferable resistance due to the *mcr-1* gene underlines the importance of microbiological and molecular surveillance and suggests the intestinal colonization as a source for dissemination. Our study contributes to the knowledge on the distribution of the *mcr-1* gene among clinical isolates in Europe.