Epidemiology and pathogenicity of Enterobacter cloacae complex clinical isolates recovered from bone and joint infections

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Background: The Enterobacter cloacae complex (ECC), composed of 13 genetic clusters, has become a major cause of opportunistic infections, especially due to its antimicrobial resistance. However, little is known about epidemiology and virulence of each cluster in human infections. The aim of the study was to determine both distribution and pathogenicity of ECC clusters associated with bone and joint infections (BJIs).

Material/methods: A collection of 47 ECC unrelated isolates recovered from BJIs between 2012 and 2015 was studied. Identification to the cluster level was obtained by partial sequencing of the hsp60 gene. All strains were characterized in vitro using the following tests: antibiotic susceptibility testing (AST), motility in 0.5% agar, biofilm formation (crystal violet assay). The virulence was assessed in vivo using the Galleria mellonella model of infection with an inoculum of ca. 2.5 ± 0.6 10⁵ CFU/larva. Survival of larvae was determined after a 24-h incubation at 37°C.

Results: Nine different ECC clusters were found in BJI specimens: C-II (3/47, 6%), C-III (13/47, 28%), C-V (1/47, 2%), C-VI (5/47, 11%), C-VII (1/47, 2%), C-VIII (15/47, 32%), C-IX (5/47, 11%), C-XI (3/47, 6%) and C-XII (1/47, 2%). No significant difference was observed between ECC clusters for AST profiles and motility abilities. By contrast, C-III and C-IX clusters were significantly more virulent than C-II, C-VIII and C-XI (P <0.01 in all cases) (Figure). Whereas no difference was found between C-III and C-IX (P = 0.284), C-III was more virulent than C-VI (P = 0.025) but it was not the case for C-IX (P = 0.176) (Figure). Interestingly, C-IX significantly produces more biofilm in vitro than all other clusters (P = 0.024), including C-III (P <0.001).

Conclusions: These results show that there is a limited diversity of ECC clusters involved in BJIs, with only two clusters (C-III and C-VIII) representing 60% of isolates. However, it appears to be a difference of virulence between them, with C-III being more pathogenic in G. mellonella. Although less encountered, C-IX seems to be as much virulent as C-III in vivo while C-IX produces more biofilm than C-III. Further investigations (such comparative genomic analysis) will be needed to understand differences of pathogenicity between clusters.
Galleria mellonella model of infection

% survival of larvae

Strains