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

e-poster **3398**

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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.

In previous studies, some authors have reported the predominance of clinical isolates belonging to clusters III, VI, and VIII from human samples (1-2). Note that, among isolates recovered from orthopedic implant isolates, Morand *et al.* have noticed that the cluster III was poorly represented (1).

The aim of the study was to determine both distribution and pathogenicity of ECC clusters associated with bone and joint infections (BJIs).

Material and methods

- ✓ A collection of 47 ECC unrelated clinical isolates recovered from BJIs between 2012 and 2015 was studied.
- ✓ Identification to the cluster level was obtained by partial sequencing of the *hsp60* gene (3).
- ✓ 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 10^5 CFU per larva. Survival of larvae was determined after a 24-h incubation at 37° C.

Results

- ❖ 9 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 (data not shown) and motility abilities (**Figure 1**).

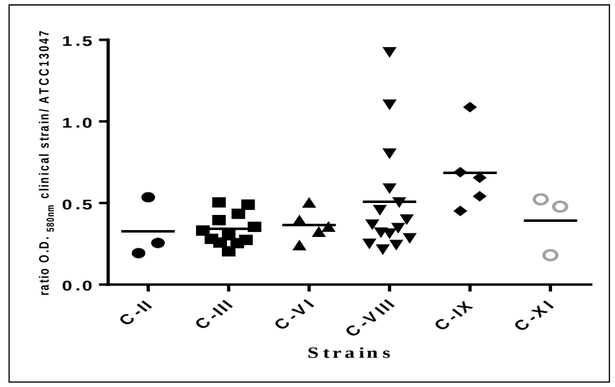

Figure 1 : Quantitative bacterial motility assay in agar (0.5%).

- ❖ Interestingly, C-IX significantly produced more biofilm in vitro than all other clusters (*P* = 0.024), including C-III (*P* <0.001) (**Figure 2**).

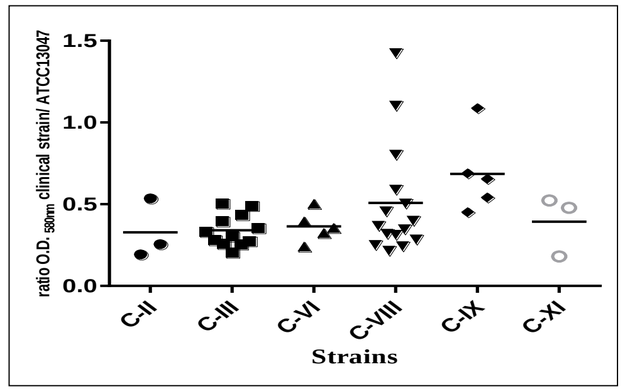

Figure 2 : Quantitative biofilm assay crystal violet).

- ❖ By contrast, C-III and C-IX were significantly more virulent than C-II, C-VIII and C-XI (*P* <0.01 in all cases) (**Figure 3**). 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 3**).

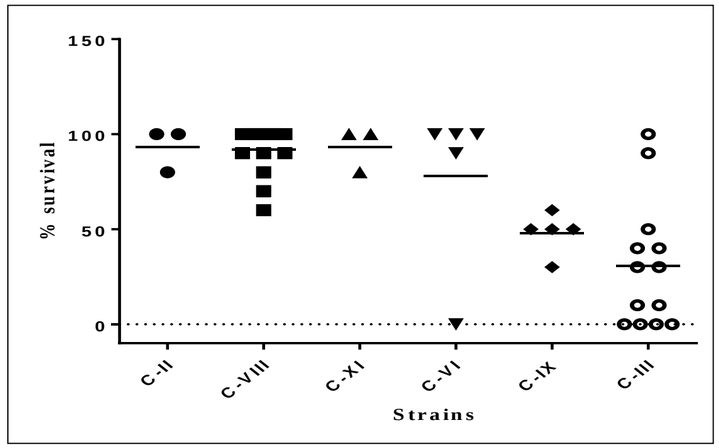

Figure 3 : *G. mellonella* virulence assay (model of infection).

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. As opposed to Morand *et al.*, we have found a more important proportion of C-III.**
- ➢ **Interestingly, it appears to have a significant 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.**
- ➢ **These results support the hypothesis that, amongst ECC isolates, C-III and C-IX are involved in BJI pathogenesis and highlight the need for a more accurate identification of clusters within the ECC.**
- ➢ **Further investigations (such comparative genomic analysis) will be required for dissecting differences of pathogenicity between clusters.**

References:

1. Morand PC, *et al*. Specific distribution within the Enterobacter cloacae complex of strains isolated from infected orthopedic implants. J Clin Microbiol. 2009 Aug;47(8):2489-95.
2. Akbari M *et al*. Particular Distribution of Enterobacter cloacae Strains Isolated from Urinary Tract Infection within Clonal Complexes. Iran Biomed J. 2016;20(1):49-55.
3. Hoffmann H, Roggenkamp A. Population genetics of the nomenspecies Enterobacter cloacae. Appl Environ Microbiol. 2003 Sep;69(9):5306-18.