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Low level OmpF porin-encoding gene expression in OXA-48 carbapenemase-producing *Enterobacter cloacae* ST-89

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Background

Nowadays, utility of carbapenems, drugs considered as "last-line" agents in therapy of infections caused by MDR pathogens is constantly decreasing. Resistance to carbapenems is reported worldwide, including enzymatic and non-enzymatic mechanisms. General outer membrane porin, OmpF was recently described as a crucial channel for β-lactam entry due to the size of the constriction zone.

We aimed to investigate the level of OmpF porin-encoding gene expression level in carbapenem-resistant OXA-48 carbapenemase-producing *Enterobacter cloacae* clinical strain relative to carbapenem-susceptible clinical strain.

Material/methods

Broth microdilution susceptibility testing and multilocus sequence typing (MLST) was performed in both carbapenem-resistant and carbapenem-susceptible *Enterobacter cloacae* strain.

Quantitative Real-Time PCR was used to investigate level of OmpF porin-encoding gene in tested *Enterobacter cloacae* strains. Total RNA was reverse transcribed with use of SuperScript-IV® enzyme, and first strand cDNA (50:1) was used for further analysis.

Determination of gene expression level in carbapenem-resistant *Enterobacter cloacae* was performed relative to carbapenem-susceptible *Enterobacter cloacae* strain. Relative difference in OmpF gene expression level was determined with use of Pfaffl efficiency calibrated $2^{-\Delta\Delta Cq}$ method with subsequent analysis of *rpoB* housekeeping gene used as internal control.

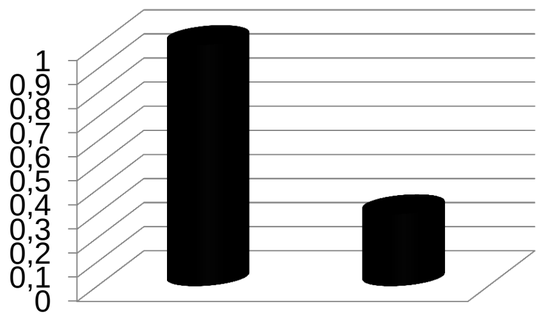
OmpF-coding transcript was amplified with use of primers pair (1.) *ompF-Forward* GAC GCA GGC TCC TTC GAC TA and (2.) *ompF-Reversed* CAA CCA GGC CGA AGA AGT TG.

Results

Carbapenem-resistant *Enterobacter cloacae* was assigned to ST-89, while wild-type clinical strain belonged to ST-335.

Broth microdillution antimicrobial susceptibility (Table 1) testing revealed *Enterobacter cloacae* ST-89 to be resistant to ertapenem, doripenem, ceftazidime, cefepime, cefotaxime, ceftriaxone, and intermediately susceptible to meropenem and imipenem (MIC 4 mg/L), while *E. cloacae* ST-335 was susceptible for all tested agents.

OmpF-gene expression level



E. cloacae ST-335 *E. cloacae* ST-89

Analysis of gene expression revealed deficiency of porin-encoding gene in carbapenem-resistant strain. Expression level of OmpF porin-encoding gene was **3,35-fold** down-regulated in carbapenem-resistant *E. cloacae* ST-89 compared to carbapenem-susceptible *Enterobacter cloacae* ST-335 strain.

Conclusions

Here we report the *E. cloacae* ST89 strain with multiple mechanisms of resistance to carbapenems. Gene expression analysis revealed contribution of decreased OmpF porin-encoding gene expression level in carbapenem-resistant *E. cloacae* ST-89 expressing acquired OXA-48 carbapenemase. The accumulation and interplay between various mechanisms of antimicrobial resistance may consequently lead to virtually untreatable infections.

Reference:

Miyoshi-Akiyama T., Hayakawa K., Ohmagari N., Shimojima M., Kirikae T. *Multilocus Sequence Typing (MLST) for Characterization of Enterobacter cloacae*. PLoS One 2013, 8 (6): e66358.

Table 1. Antimicrobial susceptibility of tested *Enterobacter cloacae* strains

	ETP *ertapenem*	**IMP** *imipenem*	**MEM** *meropenem*	**DOR** *doripenem*	**BIA** *biapenem*	**CAZ** *ceftazidime*	**FEP** *cefepime*	**CTX** *cefotaxime*	**TRX** *ceftriaxone*
***E. cloacae* ST-89**	64 mg/L	4 mg/L	4 mg/L	4 mg/L	1 mg/L	8 mg/L	256 mg/L	512 mg/L	512 mg/L
***E. cloacae* ST-335**	0,06 mg/L	0,25 mg/L	0,06 mg/L	0,03 mg/L	0,03 mg/L	0,25 mg/L	0,125 mg/L	0,25 mg/L	0,5 mg/L

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