ABSTRACT No: (9)

Study of *ompk35* and *ompk36* Expression in Carbapenem Resistant ESBL Producing Clinical Isolates Of *Klebsiella pneumoniae*

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INTRODUCTION

Carbapenem resistant extended spectrum β-lactamase (ESBL) producing *Klebsiella pneumoniae* (*K. pneumoniae*) are increasing worldwide. Carbapenem resistance (CR) has been attributed to production of carbapenemases namely class A *Klebsiella pneumoniae* carbapenemase (KPC), class B metallo-β-lactamase (MBL) or class D oxacillinases (OXA) but also to permeability barriers due to outer membrane proteins (OmpK35 and OmpK36) disruption.

OBJECTIVE

To phenotypically detect CR among ESBL producing *K. pneumoniae*, followed by the evaluation of the role of *ompK35* and *ompK36* gene expression among carbapenem resistant *K.pneumoniae* (CR-KP) isolates.

MATERIALS AND METHODS

A total of 100 *K. pneumoniae* isolates were collected from clinical specimens delivered to the Microbiology lab of Alexandria main university hospital. The isolates were identified using API 20 E system. Phenotypic detection of ESBLs was performed as guided by CLSI (2014).

Minimum inhibitory concentration (MIC) of imipenem was performed for all isolates by broth microdilution method. For CR-KP isolates, phenotypic detection of *K. pneumoniae* carbapenemase (KPC), metallo-β-lactamase (MBL) and AmpC enzymes was performed followed by Realtime qRT-PCR to detect and quantify *ompK35* and *ompK36* gene expression.

RESULTS

CR was detected in 42% of the 100 ESBL producing *K. pneumoniae* isolates where 12 isolates (12%) exhibited low level resistance (LLR) (≥4-32 µg/ml) and 30 isolates (30%) exhibited high level resistance (HLR) (>32-512 µg/ml).

All 42 isolates were KPC producers whether singly in 9/42 isolates or in combination with other enzymes: [KPC/AmpC] and [KPC/AmpC/MBL] in 13/42 isolates for each, and [KPC/MBL] in 7/42 isolates.

Reduced expression of both *ompK35* and *ompK36* was detected in (52.38%) of CR-KP isolates, while reduced expression of *ompK36* or *ompK35* alone was found in (2.38%) and (33.33%) respectively. Twenty of 42 CR-KP isolates (47.62%), showing reduced *ompK35* and *ompK36* expression, exhibited high level resistance (HLR) (>32µg/ml) to imipenem.

There was a significant correlation between reduced expression of *ompK36* and increase MIC values ($p<0.05$), while this correlation could not be established between reduced expression of *ompK35* and high MIC values.

The combined production of MBL or AmpC together with reduced expression of *ompK35* and/or *ompK36* resulted in significant increase in imipenem MIC ($p<0.05$).

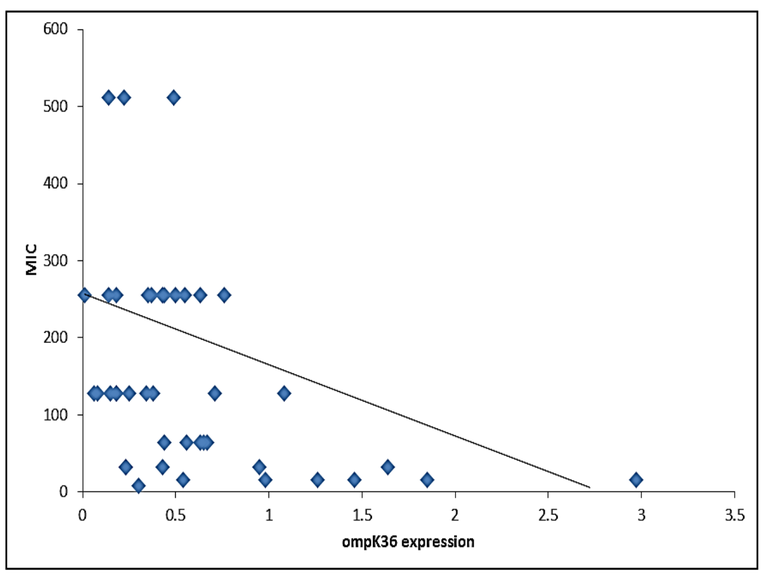

Figure 1: correlation between *ompK36* expression and MIC values. **r** = -0.446 **(p < 0.05)**

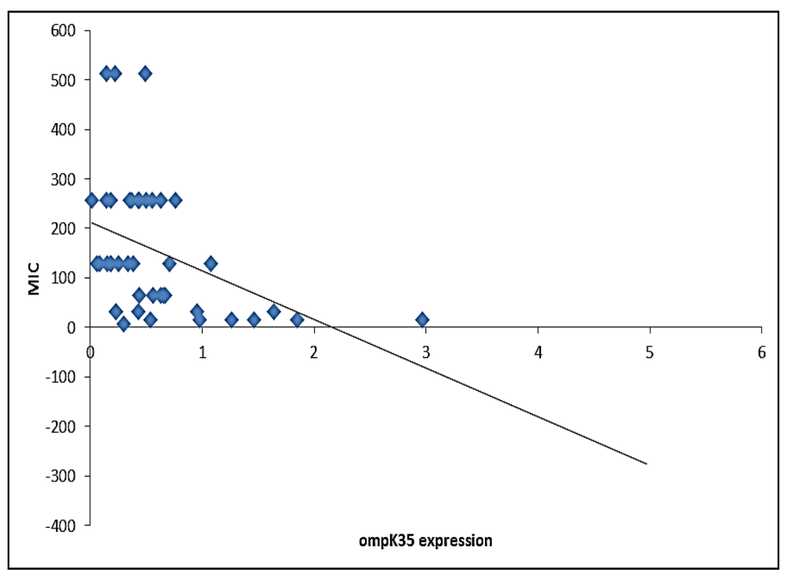

Figure 2: correlation between *ompK35* expression and MIC values. **r** = -0.162 (**p** =0.304)

CONCLUSION

The high percentage of CR calls for more rigid application of infection control measures and establishing strict antibiotic policies mainly in ICUs. Our data revealed an interplay between enzymes production and reduced expression of *ompK35* and/or *ompK36* that affected the CR level and hence the options of treatment available including combination therapies. The combined OmpK35/OmpK36 loss resulted in HLR, however OmpK36 seems to play a major role in those strains. Imipenem MIC was markedly increased among *K.pneumoniae* showing carbapenemase and/or AmpC production together with loss of OmpK35 and/or OmpK36.