

Prevalence of OmpK35 and OmpK36 porin expression in beta-lactamase and non-beta-lactamase-producing *Klebsiella pneumoniae*

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Background: The aims of this study were to confirm the presence of OmpK35 and OmpK36 in extended-spectrum beta-lactamase-producing and nonextended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and to determine the relationship between porin expression and resistance to third-generation cephalosporins.

Methods: Fifty-two *K. pneumoniae* isolates were obtained and analyzed for extended-spectrum beta-lactamase and for OmpK35 and OmpK36.

Results: Twenty-two (42.3%) isolates of *K. pneumoniae* were extended-spectrum beta-lactamase producers. The OmpK35 profile in *K. pneumoniae* producing extended-spectrum beta-lactamase showed the presence of porin protein in ceftazidime-sensitive *K. pneumoniae* (six isolates), and the OmpK36 profile in *K. pneumoniae* producing extended-spectrum beta-lactamase revealed isolates sensitive to cefotaxime (n = 8) and ceftriaxone (n = 6). All nonextended-spectrum beta-lactamase-producing *K. pneumoniae* showed the presence of OmpK35 and OmpK36 porin proteins.

Conclusion: The presence of OmpK35 is mostly related to ceftazidime susceptibility and less to cefotaxime and ceftriaxone susceptibility, while OmpK36 expression is seen more often in cefotaxime-sensitive isolates. OmpK35 and OmpK36 indicate nonextended-spectrum beta-lactamase producing strains, and their presence is important when selecting an antimicrobial agent.

Keywords: *Klebsiella pneumoniae*, extended-spectrum beta-lactamase producing strains, porin expression

Introduction

Because of development of plasmids producing extended-spectrum beta-lactamase (ESBL), *Klebsiella pneumoniae* is resistant to a number of broad-spectrum antibiotics, including cephalosporins. Therefore, treatment of infections caused by bacteria producing ESBL is problematic.^{1,2} The first report of ESBL-producing *K. pneumoniae* occurred in 1983 in Germany.¹ Majiduddin et al then classified these enzymes based on their sequence similarity and catalytic mechanism into four main groups, ie, classes A, B, C, and D.³

K. pneumoniae produces two major porins, OmpK35 and OmpK36, and the quiescent porin OmpK37. Details on OmpK36 and OmpK37 have been reported previously.⁴ Most clinical isolates of *K. pneumoniae* lacking ESBL express both OmpK35 and OmpK36 porins, while most ESBL-expressing *K. pneumoniae* clinical isolates produce only OmpK36.⁵ Until now, the few clinical isolates lacking both OmpK35 and OmpK36 have been ESBL-producing strains.⁶

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Loss of OmpK36 is associated with cefoxitin resistance and increased resistance to oxyimino and zwitterionic cephalosporins in strains producing ESBL and to carbapenem resistance in strains producing plasmid-mediated AmpC-type beta-lactamase.⁷ Loss of OmpK36 also results in a moderate increase in fluoroquinolone resistance in strains with altered topoisomerases and/or active efflux of quinolones. Preliminary results indicate that OmpK35 allows efficient penetration of cefoxitin, cefotaxime, and carbapenem, but there has been some controversy on the role of this porin in cephalosporin penetration in *K. pneumoniae*.⁸ Detailed studies of the importance of OmpK35 in antimicrobial resistance are lacking. The aims of this study were to determine the presence of OmpK35 and OmpK36 in ESBL-producing and non-ESBL-producing *K. pneumoniae* and to find a relationship between porin expression and resistance to third-generation cephalosporins.

Materials and methods

Bacterial strains

Fifty-two *K. pneumoniae* isolates were obtained from March 2010 to August 2010 at Milad Hospital, Tehran, Iran. All isolates were from patients with urinary tract infection. The sensitivity of the isolates to third-generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone) and to second-generation cephalosporin (cefoxitin) and a carbapenem (imipenem) was determined by the disc diffusion method (each antibiotic 30 µg/disc).⁹ The results were interpreted as per National Committee for Clinical Laboratory Standards recommendations.⁶ *Escherichia coli* ATCC 25922 was used as a quality control.

ESBL detection by double-disc synergy test

Isolates with resistance to or decreased susceptibility (intermediate by National Committee for Clinical Laboratory Standards criteria) to any of the third-generation cephalosporins were subjected to the standard double-disc synergy test.¹⁰ Because the isolates in the present study were found to be resistant to at least one of the three third-generation cephalosporins, they were all tested for ESBL production using this test.

Analysis of outer membrane proteins

Bacterial cells were grown in nutrient beef extract broth 3 g and Proteose Peptone 3.5 g (both from Difco Laboratories Inc, Detroit, MI) in 1 L of distilled water to increase OmpK35 expression. Cells in the logarithmic phase were lysed by

sonication and the cell membranes were recovered by ultracentrifugation. Outer membrane proteins were obtained after treatment of cell membranes with sodium lauryl sarcosinate (Sigma-Aldrich, St Louis, MO) and subsequent ultracentrifugation. Outer membrane protein profiles were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 11% acrylamide, 0.5% bis-acrylamide, and 0.1% sodium dodecyl sulfate in the running gel.¹¹

Statistical analysis

Chi-square testing using SPSS version 16 (SPSS Inc, Chicago, IL) was undertaken with appropriate correction for the observation and for the significance between ESBL production and OmpK35/36 expression $P \geq 0.05$ was considered to be statistically significant.

Results

Phenotypic ESBL screening and confirmation using the double-disc synergy test showed that 22 (42.3%) isolates of *K. pneumoniae* were ESBL producers and 30 (57.7%) isolates were non-ESBL producers. Of the 22 *K. pneumoniae* isolates, 16 (72%), 14 (63.6%), and 16 (72%) isolates were resistant to ceftazidime, cefotaxime, and ceftriaxone, respectively.

The OmpK35 profile in *K. pneumoniae* producing ESBL showed the presence of porin protein in six (27.3%) ceftazidime-sensitive *K. pneumoniae* isolates. OmpK35 was not expressed in cefotaxime-susceptible isolates but was expressed in four (18.2%) ceftriaxone-sensitive isolates. A significant difference was found between the antimicrobial panels for ceftazidime ($P < 0.01$), cefotaxime ($P < 0.003$), and ceftriaxone ($P < 0.013$) and OmpK35 in ESBL-positive strains.

The OmpK36 profile in *K. pneumoniae* producing ESBL was identified in all isolates sensitive to cefotaxime (27.3%) and ceftriaxone (36.4%) but was only seen in two (9%) *K. pneumoniae* isolates sensitive to ceftazidime. No significant difference was observed between OmpK36 and the antimicrobial panel for ceftazidime ($P < 0.13$), while a significant difference occurred between the antimicrobial panel of ceftriaxone ($P < 0.017$) and cefotaxime ($P < 0.003$) with OmpK36 (Table 1). All non-ESBL-producing *K. pneumoniae* showed OmpK35 and OmpK36 protein expression.

Discussion

OmpK35 and OmpK36 provide a channel enabling a wide range of antibiotics to penetrate the cell wall of *K. pneumoniae*, and this was initially shown by the cloning

Table 1 Third-generation cephalosporins pattern and presence of OmpK in *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase

<i>K. pneumoniae</i>	Ceftazidime	Cefotaxime	Ceftriaxone	OmpK35	OmpK36
K1	S	R	S	P	P
K2	S	R	S	P	P
K3	S	R	R	P	N
K4	S	R	R	P	N
K5	S	R	R	P	N
K6	S	R	R	P	N
K7	R	S	R	N	P
K8	R	S	R	N	P
K9	R	S	R	N	P
K10	R	R	S	P	P
K11	R	S	S	N	P
K12	R	S	R	N	P
K13	R	S	R	N	P
K14	R	S	R	N	P
K15	R	S	R	N	P
K16	R	R	R	N	N
K17	R	R	R	N	N
K18	R	R	R	N	N
K19	R	R	R	N	N
K20	R	R	R	N	N
K21	R	R	S	P	P
K22	R	R	S	N	P

Abbreviations: R, resistance; S, sensitive; P, positive; N, negative.

and ectopic expression of OmpK35 or OmpK36 in a strain lacking both OmpK35 and OmpK36.¹² Porins play a crucial role in the interaction between bacteria and the environment. In addition, and probably as a consequence, porins are present in large amounts in the outer membrane of Gram-negative bacteria. Since the identification of the major porins, OmpC and OmpF, in *E. coli*, a large number of OmpC-type or OmpF-type porins have been described in other enterobacterial species. We have reported the existence of two major porins, OmpK36 and OmpK35, in *K. pneumoniae*. These porins are homologous to OmpC and OmpF, and are expressed in large amounts in most *K. pneumoniae* clinical isolates independent of the isolation source.⁵ We have focused our attention on the role of these porins in penetration of the cell wall by antibiotics. As has been reported for other species, the absence of porin is an important cause of resistance to some antimicrobials, particularly beta-lactam antibiotics.¹³

As a result of these investigations, we isolated clinical strains resistant to most of the beta-lactam antibiotics currently used to treat *K. pneumoniae* infections. These isolates had a characteristic in common, ie, they simultaneously lacked expression of porins, OmpK35 and OmpK36. Most clinical isolates of *K. pneumoniae* lacking ESBL express both OmpK35 and OmpK36 porins. Our results indicate that the

presence of OmpK35 is most related to ceftazidime susceptibility and less to cefotaxime and ceftriaxone susceptibility, while OmpK36 expression is more commonly observed in cefotaxime-sensitive isolates.

Porins are important for bacterial survival because of their role in the exchange of substances, including nutrients and toxic metabolites. Previous studies have shown that few clinical ESBL-producing *K. pneumoniae* isolates lack both OmpK35 and OmpK36, although strains lacking both porins exhibit higher antibiotic resistance than strains that express only one or both porins.¹⁴ These results suggest that OmpK35 and OmpK36 play dual roles in *K. pneumoniae* infection.

In conclusion, the results indicate that the presence of OmpK35 is mostly related to ceftazidime susceptibility and less to cefotaxime and ceftriaxone susceptibility, while OmpK36 expression is more commonly observed in cefotaxime-sensitive isolates. Our results also show that OmpK35 and OmpK36 are found in non-ESBL-producing strains, and their presence is important when selecting an antimicrobial agent.

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Disclosure

The authors report no conflicts of interest in this work.

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