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RESISTANCE OF *KLEBSIELLA PNEUMONIAE* CLINICAL ISOLATES: LINKAGE OF OUTER MEMBRANE PROTEINS (OMPS) WITH PRODUCTION ESBLS

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ABSTRACT

Three isolates of *Klebsiella pneumoniae*, collected from the University Hospital in Fortaleza, Brazil, were analyzed to determine their resistance to multiple antibiotics. The results of this study showed that the resistance of the clinically isolated bacteria is associated with the production of extended-spectrum beta-lactamases (ESLBs) and loss of outer membrane proteins.

Key words: *Klebsiella pneumoniae,* β-lactamase and porin.

Klebsiella pneumoniae (*Enterobacteriaceae*) is an opportunist human pathogen that accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicaemia, and soft tissue infections. Due to its ability to spread rapidly in a hospital environment and resistance to multiple drugs, this strain is for nosocomial outbreaks world-wide, generally associated with high morbidity and mortality (5,6).

One of the most prevalent mechanisms of bacterial resistance among Gram-negative bacteria is the production of β-lactamases. Extended-spectrum β-lactamases (ESBLs) are a group of clinically very important β-lactamases because they are able to hydrolyze the extended-spectrum cephalosporins (ceftazidime, cefotaxime, ceftriaxone) and monobactans (aztreonam) (2). Frequently, bacterial species will not only possess a β-lactamase, but also exhibit porin deletion and thus resistance derives from a synergy between reduced permeability and β-lactamase activity (8). The outer-membrane proteins (OMPs) of *Enterobacteriaceae* have medical importance because its constituents play major roles in the permeability of antimicrobial agents and substrates, and in interactions with the host defense mechanisms (3).

The aim of the present study was to evaluate the relationship between the resistances of ESBL-expressing *K. pneumoniae* clinical isolates and lost outer membrane proteins.

The *K. pneumoniae* isolates were collected from hospitalized patients in the Walter Cantídio University Hospital, Fortaleza/Brazil, in June of 2005. The strains were identified by standard biochemical methods and *K. pneumoniae* ATCC 4352 and *E.coli* ATCC 25922 was used as the reference strain. The strains were maintained as frozen stocks at -70ºC in the presence of 15% of glycerol and cultured in brain heart infusion (BHI) agar (Difco) for 24 hours at 37ºC for later analysis. Antimicrobial susceptibility to cephalotin, cefoxitin,

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ceftriaxone, cefotaxime, amoxillin/clavulanate cefepime and aztreonam was performed by disk diffusion method according to Clinical and Laboraty Standards Institute (CLSI) recommendations (7). All tests were carried out three times. The isolates were further examined by the double-disk synergy test for ESBLs detection as previously described by Vercauteren *et al*. (9), based on the inhibition toward the disk containing the β-lactamase inhibitor clavulanate.

Electrophoretic analysis of *K. pneumoniae* OMPs was carried out using the buffer system of Laemmli (4). Bacterial cultures were grown at 37° C overnight in BHI agar medium. The cells were collected by centrifugation (13000g for 3 min.) of 1 mL of culture and suspended in 100 µl of sample buffer. The cells suspensions were boiled at 100°C for 10 minutes. In addition, 20 µl of the protein were used for SDS-PAGE on a vertical slab gel, containing 4% stacking gel and a 17% separating gel. Protein bands were visualized by Coomassie brilliant blue staining. Molecular weights were estimated by comparison with the protein molecular weight markers MW-SDS-70L (Sigma-Aldrich Co., USA), ranging in size from 14,4-94,0 kDa. The protein concentration of bacterial suspension was determined by the Bradford essay (1).

Table 1 displays the results of antimicrobial susceptibility of *K. pneumoniae* isolates. Kp 3, Kp 6 and Kp 8 were resistant to third and fourth generation cephalosporins and monobactans. Application of the double-disk synergy test procedure also showed that these isolate ESBL production. This data confirmed the observation of Lopes et al., (5) that *K. pneumoniae* is resistant to a number of antibiotics, mainly extended-spectrum cephalosporins and penicillins, due to acquisition of plasmids that encode for the production of extended-spectrum β-lactamases.

Table 1. Antimicrobial susceptibility of *K. pneumoniae* clinical isolates and reference strain

Strains	Resistance phenotypes
E. coli (ATCC 25922)	-
Kp (ATCC 4352)	-
Kp 8	KF, CRO, ATM, AMC/CAC, CTX,
Kp 6	KF, CRO, FOX, ATM, FEP
Kp 3	KF, CRO, FOX, ATM, FEP, AMC/CAC, CTX,

- not resistance; KF-cephalotin, FOX-cefoxitin, CRO-ceftriaxone, CTX-cefotaxime, FEP-cefepime, ATM-aztreonam, AMC/CAC-amoxicilin/clavulanate.

SDS-PAGE analysis (Fig. 1) of the *K. pneumoniae* isolates revealed several proteins bands in the 30 to 66 kDa range. The results of this study indicate that Kp3, Kp 6 and Kp 8 strains, resistant to aztreonam and all cephalosporins (Table 1) to loss one porin. However, we can say that this porin is 30-43 kDa range, suggesting that it may be OmpK35 or OmpK36. It has been clearly demonstrated that porin deficiency contributes to increasing the level of resistance to ESBLs-producing strains (3).

In conclusion, this data suggests that resistance to *K. pneumoniae* clinical isolates is due to the association between extended-spectrum beta-lactamases (ESLBs)-producing with lost outer membrane proteins. Further studies, using techniques of molecular biology will be developed with the intention of confirming production ESBLs.

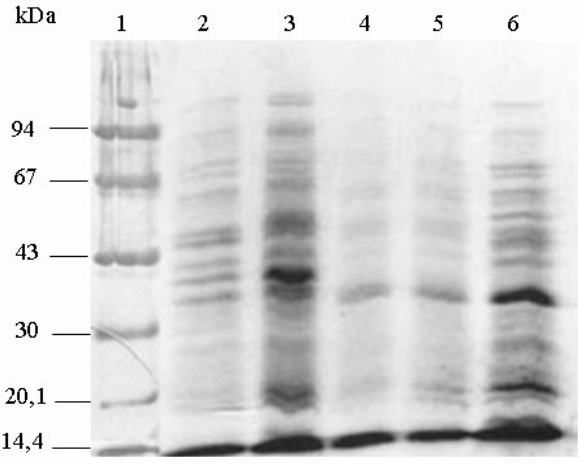

Figure 1. SDS-PAGE analysis of *Klebsiella pneumoniae* clinical isolates. Molecular sizes (lane 1), *E. coli* ATCC 25922 (lane 2), Kp ATCC 4352 (lane 3), Kp 08 (lane 4), Kp 06 (lane 5), Kp 03 (lane 6)

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