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RESEARCH NOTE

Evaluation of differential gene expression in susceptible and resistant clinical isolates of *Klebsiella pneumoniae* by DNA microarray analysis

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ABSTRACT

DNA microarray technology was used to evaluate differential gene expression in a susceptible *Klebsiella pneumoniae* isolate and a resistant clinical derivative. Nineteen genes were up-regulated in the resistant isolate when compared with the susceptible isolate. An ABC transporter-related gene, *ycjV*, was strongly over-expressed, suggesting the existence of a novel active efflux mechanism. Approximately half of the up-regulated genes coded for ribosomal proteins, or proteins involved in tRNA metabolism. Among 33 down-regulated genes, almost one-third were related to nitrogen metabolism. A possible role of fitness in the development of antimicrobial resistance is suggested.

Keywords Antimicrobial resistance, efflux pumps, gene regulation, *Klebsiella pneumoniae*, microarray, transcriptional analysis

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Klebsiella pneumoniae is an opportunistic human pathogen causing a wide range of infections, including urinary tract infection, bacteraemia and pneumonia. Expanded-spectrum cephalosporins are effective in the management of *K. pneumoniae* infections; however, increased use of these agents has resulted in the emergence of extended-spectrum β-lactamase-producing strains that have become an increasingly serious problem worldwide [1,2]. In addition to these plasmid-encoded β-lactamases, various other mechanisms of resistance to cephalosporins and other antimicrobial agents have been described in *K. pneumoniae* [3–7]. These observations indicate that antimicrobial resistance in *K. pneumoniae* is a complex and multifactorial phenomenon, which may also depend on the up-regulation or down-regulation of as yet unknown genes.

DNA microarrays offer a useful means of global genome analysis, since the expression of thousands of genes can be studied in a single experiment. In the case of *K. pneumoniae*, microarrays containing *Klebsiella* DNA are not yet available. However, microarrays containing *Escherichia coli* DNA have been used successfully to analyse the genome of *K. pneumoniae*, as most (70%) *E. coli* genes are also present in the *Klebsiella* chromosome [8]. The present study used DNA microarray technology to identify differentially expressed genes associated with antimicrobial resistance by comparing the transcriptional profile of the susceptible isolate CSUB10S with that of its resistant derivative CSUB10R, isolated from the same patient [9]. Resistance mechanisms in CSUB10R include porin deficiency, β-lactamase production, *gyrA* mutation and active efflux [5,7,9]. The clonal relationship between CSUB10S and CSUB10R has been demonstrated by pulsed-field gel electrophoresis [9]. For strain CSUB10R, the MICs of cefotetan, cefepime, ceftazidime and aztreonam were >256 mg/L, and the MICs of cefoxitin and ciprofloxacin were 128 mg/L and 4 mg/L, respectively. The entire microarray procedure was performed as described previously [8]. Dye swap was performed in the present analysis, using Cy3 dye for CSUB10S and Cy5 dye for CSUB10R in the first two experiments, and Cy5 dye for CSUB10S and Cy3 dye for CSUB10R in the remaining experiments. Similar results were obtained in all cases. Statistical analysis showed that genes with $\log_2$ ratio values >0.75 were over-expressed in CSUB10R, whereas those with $\log_2$ ratio values <0.79 were down-regulated ($p < 0.01$).

Applying the above criteria, the resistant *K. pneumoniae* strain CSUB10R over-expressed 19 genes when compared with the susceptible strain CSUB10S (Table 1). Among these genes, *ycjV* was strongly over-expressed (> two-fold). This gene encodes the ATP-binding protein of an ABC transport system. It has been shown that CSUB10R expresses an active efflux mechanism [5]; however, transcriptional analysis did not detect changes in the expression of the main (AcrAB–TolC) efflux systems in *E. coli* and *K. pneumoniae* [10], suggesting that other transport systems or genes, e.g., *ycjV*, may be responsible for this efflux.

Among the 19 genes with increased expression levels in CSUB10R, >50% were ribosomal protein genes (*rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplW*, *rpsE*, *rpsJ*, *rpsK*, *rpsS*) and genes involved in tRNA metabolism (*trmD*, *glyS*). Other over-expressed genes, related to nucleic acid metabolism, were *nrdD* and *rho*.

Gene members of operons, including *kdgR* and *eda*, were coordinately regulated, supporting the congruency of the results. *kdgR* is an activator of *eda*, a gene involved in gluconate metabolism, as is the case for *pykF*, another over-expressed gene in CSUB10R. The *ycjV* gene has been reported to be involved in the metabolism and transport of carbohydrates. Although the natural function of the AcrAB efflux pump is not related to antimicrobial resistance, its over-expression results in the expulsion of antimicrobial agents. It may be that over-expression of the *eda* and *pykF* genes is linked to that of *ycjV*.

The 33 genes with decreased relative expression levels in CSUB10R are also listed in Table 1, and were clearly more numerous than the over-expressed genes. Among these genes, 30% were related to nitrogen metabolism and 36% corresponded to open reading frames with unknown functions. Genes associated with nitrogen metabolism were *narG*, *narH*, *narI*, *narJ*, *narK*, *narL*, *narY*, *narZ*, *nirB* and *nirD*. Additionally, *allD* codes for an ureidoglycolate dehydrogenase involved in nitrogen assimilation from alantoin in *E. coli* [11], and *clpB* is activated in the nitrogen-fixing process [12]. It may be that, in agreement with the fitness concept [13,14], these genes were not essential for survival, and that they are not expressed by *K. pneumoniae*. This supports a