Reduced susceptibility to carbapenems in *Klebsiella pneumoniae* clinical isolates associated with plasmid-mediated β-lactamase production and OmpK36 porin deficiency

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Two carbapenem-non-susceptible *Klebsiella pneumoniae* isolates, Z2554 and Z2110, were collected from a hospital in China and analysed by PFGE. *K. pneumoniae* Z2554 and Z2110 were genetically unrelated and showed resistance to ertapenem, and reduced susceptibility to imipenem and meropenem. Analysis of their β-lactamases indicated that *K. pneumoniae* Z2554 produced TEM-1 and CTX-M-14 β-lactamases, whilst Z2110 produced a plasmid-mediated AmpC β-lactamase, DHA-1, in addition to TEM-1 and CTX-M-14. SDS-PAGE analysis of the outer-membrane proteins (OMPs) revealed that both isolates lacked an OMP of ~39 kDa (OmpK36), whilst Z2110 had an additional protein with an approximate molecular mass of 26 kDa. Analysis of the OMP-encoding genes demonstrated that the *ompK35* sequence of *K. pneumoniae* Z2554 and Z2110 contained a number of silent mutations. In *ompK36*, several insertions and deletions of short DNA fragments (1–6 bp) were detected in both isolates. The N-terminal sequence of the ~26 kDa protein band identified in Z2110 had no similarity to the sequence of OmpK36. Instead, it shared high similarity with hypothetical protein KPN _03267 originating from *K. pneumoniae* subsp. *pneumoniae* MGH 78578. It was concluded that β-lactamase production combined with OmpK36 deficiency results in ertapenem resistance, and reduced imipenem and meropenem susceptibility, in *K. pneumoniae* Z2554 and Z2110. OmpK36 may play an important role in the resistance or reduced susceptibility to carbapenems in *K. pneumoniae* producing AmpC, extended-spectrum β-lactamase or broad-spectrum β-lactamase.

**INTRODUCTION**

*Klebsiella pneumoniae* is one of the most important pathogens that causes nosocomial infections. Due to the extensive use of extended-spectrum cephalosporins, extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* has become an increasingly serious problem. Carbapenems are commonly used to treat serious infections caused by multidrug-resistant Gram-negative bacteria, especially strains producing high levels of AmpC cephalosporinases or ESBLs. Although carbapenem-resistant *K. pneumoniae* is uncommon, a number of *K. pneumoniae* isolates have been reported to be resistant to carbapenems mediated by metallo-β-lactamases in Singapore, Brazil, Turkey and Taiwan, including IMP-1 and IMP-8 (Aktas *et al.*, 2006; Koh *et al.*, 1999; Lincopan *et al.*, 2005; Yan *et al.*, 2001). Recently, the number of *K. pneumoniae* isolates producing KPC-type carbapenemases has increased dramatically (Smith Moland *et al.*, 2003; Wei *et al.*, 2007; Woodford *et al.*, 2004; Yigit *et al.*, 2001).

A combination of high-level production of AmpC β-lactamases (e.g. ACT-1, CMY-4 and DHA-1) or SHV-2 together with porin loss can result in resistance or reduced susceptibility to carbapenems in *K. pneumoniae* (Bradford *et al.*, 1997; Cao *et al.*, 2000; Crowley *et al.*, 2002; Lee *et al.*, 2007a). Recently, an outbreak of imipenem-non-susceptible *K. pneumoniae*, caused by the production of CMY-2 and DHA-1 together with OmpK35 and OmpK36 porin loss, was reported in Korea (Lee *et al.*, 2007b).

Studies have been conducted to elucidate the mechanism of resistance to carbapenems in *K. pneumoniae* as a result of a deficiency of porins in combination with the production of β-lactamases. Lee *et al.* (2007a) reported that strains that are deficient in both OmpK35 and OmpK36 have resistance to carbapenems in the presence of β-lactamases. However, in a strain where only OmpK35 was deficient and OmpK36 was expressed, the strain maintained its suscept-
Reduced carbapenem susceptibility in K. pneumoniae

METHODS

Bacterial strains and antimicrobial susceptibility testing. Two carbapenem-non-susceptible K. pneumoniae strains, Z2554 and Z2110, were isolated from the sputum of two patients in November 2006 and identified using a Vitek system (bioMérieux). One patient was hospitalized in the oncology ward due to extensive abdominal cavity metastasis after a radical operation for gastric cancer. Cefoperazone/sulbactam and ciprofloxacin (2.0 g, intravenously (i.v.), twice a day) and ciprofloxacin (0.4 g, i.v., twice a day) were administered for pulmonary infection for 2 weeks and then replaced with ceftiminox (2.0 g, i.v., twice a day) and gatifloxacin (0.4 g, i.v., once a day). Two days later, K. pneumoniae Z2554 was isolated. The other patient was hospitalized in the respiratory ward for severe pneumonia and treated with multiple antimicrobials, including ceftriaxone (2.0 g, i.v., once a day) and ciprofloxacin (0.5 g, i.v., once a day) for 5 days, panipenem (1.0 g, i.v., every 12 h) and mycymycin (500 000 U, orally, three times a day) for 2 weeks, and ceftiminox (2.0 g, i.v., twice a day) and gatifloxacin (0.4 g, i.v., once a day) for 5 days, after which K. pneumoniae Z2110 was isolated.

The MICs of 16 antimicrobial agents were determined using an agar dilution method according to Clinical and Laboratory Standards Institute recommendations (CLSI, 2006). The antimicrobial agents were: piperacillin, cepoxatine, gentamicin, tetracycline, sulfonamide (Sigma), imipenem, etarpenem, cefotaxin (Merck), meropenem (Dainippon Sumitomo Pharma), piperacillin/tazobactam (Wyeth Holdings), cefoperazone/sulbactam (Pfizer), cefazidime (GlaxoSmithKline), ceftriaxone (Roche), aztreonam, ceftazime (Bristol-Meyers Squibb) and ciprofloxacin (Bayer).

PFGE. PFGE of the two K. pneumoniae isolates was performed according to the protocols of PulseNet (http://www.cdc.gov/pulsenet/protocols.htm) in a Rotaphor system 6.0 instrument (Whatman Biometra). The XbaI restriction patterns of their genomic DNA were analysed and interpreted according to the criteria of Tenover et al. (1995).

Conjugation experiment. A conjugal transfer experiment was carried out in mixed broth cultures. Rifampicin-resistant Escherichia coli EC600 (LacZ+, NalR, RifR) was used as the recipient. E. coli transconjugants were selected on Mueller–Hinton agar containing rifampicin (700 μg ml⁻¹) and imipenem (0.5 μg ml⁻¹), or rifampicin (700 μg ml⁻¹) and cefoxitin (16 μg ml⁻¹) (all from Sigma). The selected colonies were picked and identified using a Vitek system. Plasmid DNA of the E. coli transconjugant, K. pneumoniae clinical isolates and E. coli V517 (Macrina et al., 1978) were obtained using an AxyPrep plasmid miniprep kit (Axygen Scientific).

IEF of β-lactamase. Crude β-lactamase preparations were obtained by ultrasonic treatment of bacterial cells. IEF was carried out on a PhastGel polyacrylamide gel (pH 3–9; Amersham Biosciences) using a PhastSystem (Pharmacia Biotech) and the method of Mathew et al. (1975). β-Lactamase activity was visualized by staining the gel with nitrocefin (Oxoid). Isoelectric points (pIs) were determined after comparison with the known β-lactamases TEM-28 (pI 6.1), SHV-7 (pI 7.6) and ACT-1 (pI 9.0).

PCR amplification and DNA sequence analysis of bla genes. Plasmids from K. pneumoniae Z2554 and Z2110 and the E. coli transconjugant were used as templates. The primers used to amplify blaTEM, blaCTX-M and blaDHA were as described by Yan et al. (2002) and Yu et al. (2007). The reaction was conducted in a GeneAmp PCR system 9600 thermal cycler (Applied Biosystems). PCR products were purified and sequenced directly. DNA sequences were compared with reported nucleotide sequences in GenBank.

Analysis of OMPs. OMPs were obtained as described by Hernández-Alés et al. (1999). Strains were grown in Mueller–Hinton broth overnight at 37 °C with shaking. OMP profiles were determined by SDS-PAGE using 11.6% acrylamide/0.4% bisacrylamide/0.1% SDS, pH 8.3, at 110 V for 1 h. Purified OMPs were run on a PhastSystem (Pharmacia Biotech) with 25% SDS-PAGE using 11.6% acrylamide/0.4% bisacrylamide/0.1% SDS, pH 8.3, at 110 V for 1 h. Isoelectric points (pIs) were determined after comparison with the known OMPs TEM-28 (pI 6.1), SHV-7 (pI 7.6) and ACT-1 (pI 9.0).

N-terminal sequence analysis. The N-terminal sequence of the protein identified by SDS-PAGE of the OMPs from Z2110 was determined. The protein was transferred to a PVDF membrane after SDS-PAGE in a CAPS buffer system. The membrane was stained with Coomassie brilliant blue and the band corresponding to a protein of ~26 kDa was excised and used for N-terminal amino acid sequence analysis. The sequence was determined using an Applied Biosystems protein sequencer (Procise 492 cLC). The sequence of 15 aa was determined. The sequence was used to search and compare with sequences in GenBank.

RESULTS AND DISCUSSION

Antimicrobial agent susceptibility and PFGE profiles

The MICs of 16 antimicrobial agents are presented in Table 1. K. pneumoniae Z2554 and Z2110 showed...
resistance to ertapenem and various levels of reduced susceptibilities to imipenem and meropenem. *K. pneumoniae* Z2554 was highly resistant to penicillins, cephalosporins, aztreonam and quinolones, but remained susceptible to aminoglycosides. *K. pneumoniae* Z2110 was resistant to all of the tested antimicrobial agents. The MIC of cefoxitin for *K. pneumoniae* Z2210 was much higher than that for Z2554, although the MICs of the β-lactamase inhibitors, cephalosporins and aztreonam were lower. The MICs against ertapenem, imipenem and meropenem were also determined in the presence of β-lactamase inhibitors. As shown in Table 1, the MICs were significantly reduced by these inhibitors.

*K. pneumoniae* Z2554 and Z2110 had different PFGE patterns (Fig. 1), indicating that they were genetically unrelated.

### Transfer of antimicrobial agent resistance and plasmid analysis

For *K. pneumoniae* Z2554 and Z2110, no colonies grew on Mueller–Hinton agar containing rifampicin and imipenem.

### Table 1. Antimicrobial susceptibility patterns of the *K. pneumoniae* isolates and the *E. coli* transconjugant

<table>
<thead>
<tr>
<th>Antimicrobial agent*</th>
<th>MIC (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>K. pneumoniae</em> Z2554</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5</td>
</tr>
<tr>
<td>Imipenem + CLA</td>
<td>0.25</td>
</tr>
<tr>
<td>Imipenem + CLO</td>
<td>0.5</td>
</tr>
<tr>
<td>Imipenem + CLA + CLO</td>
<td>≤0.125</td>
</tr>
<tr>
<td>Meropenem</td>
<td>8</td>
</tr>
<tr>
<td>Meropenem + CLA</td>
<td>2</td>
</tr>
<tr>
<td>Meropenem + CLO</td>
<td>8</td>
</tr>
<tr>
<td>Meropenem + CLA + CLO</td>
<td>1</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>16</td>
</tr>
<tr>
<td>Ertapenem + CLA</td>
<td>4</td>
</tr>
<tr>
<td>Ertapenem + CLO</td>
<td>8</td>
</tr>
<tr>
<td>Ertapenem + CLA + CLO</td>
<td>1</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>256</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>32</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>256</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cefoperazone/sulbactam</td>
<td>256</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>8</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>16</td>
</tr>
</tbody>
</table>

NT, Not tested.

*CLA, Clavulanic acid (concentration fixed at 4 µg ml⁻¹); CLO, cloxacillin (concentration fixed at 200 µg ml⁻¹).*

![Fig. 1. PFGE fingerprinting of *K. pneumoniae* Z2554 and Z2110 after XbaI digestion. Lanes: 1, Z2554; 2, Z2110.](image-url)
Transfer of cefoxitin resistance from *K. pneumoniae* Z2110 to *E. coli* EC600 was successful. The transfer frequency was approximately $10^{-5}$ to $10^{-6}$ (60–100 transconjugants per $10^7$ recipients). The *E. coli* transconjugant was resistant to cefoxitin and gentamicin, and moderately resistant to penicillins, but was susceptible to cephalosporins, aztreo- nam, quinolones and carbapenems (Table 1).

The plasmid profiles (Fig. 2) indicated that *K. pneumoniae* Z2554 and Z2110 harboured several plasmids. The *E. coli* transconjugant acquired a plasmid of ~50 kb from *K. pneumoniae* Z2110. This transfer into *E. coli* EC600 by conjugation resulted in resistance to cefoxitin and *K. pneumoniae* Z2554 and Z2110 harboured several plasmids. The plasmid profiles (Fig. 2) indicated that *K. pneumoniae* Z2554 and Z2110 showed various levels of resistance to carbapenems but did not produce any type of carbapenemase, indicating that other carbapenem resistance mechanisms, such as alteration in outer-membrane permeability or an efflux pump, might be involved. However, as the efflux mechanism is unlikely to be involved in the resistance of enterobacteria, we focused on an alteration in the OMPs. SDS-PAGE analysis of the OMPs was performed, and the result revealed loss of an OMP of ~39 kDa in both *K. pneumoniae* Z2554 and Z2110 compared with *K. pneumoniae* ATCC 13883 (Fig. 3). It was uncertain whether the 39 kDa OMP was OmpK36 or OmpK35, as in some strains OmpK36 migrates faster than OmpK35 (Hernández-Allés et al., 1999). The ompK35 gene was amplified, sequenced and aligned with that of *K. pneumoniae* KT755 (GenBank accession no. AJ011501) (Domènech-Sánchez et al., 2003), and point mutations were found at nt 420, 474, 537 and 786 on the nucleotide sequence in both *K. pneumoniae* Z2554 and Z2110, and an additional point mutation was present in Z2554 at nt 249. However, point mutations in the ompK35 gene were all silent and did not lead to amino acid sequence changes. Comparing the ompK36 gene of *K. pneumoniae* Z2554 and Z2110 with that of *K. pneumoniae* C3 (GenBank accession no. Z33506; Alberti et al., 1995), several small DNA fragment insertions and a number of point mutations were observed. The sequence of the ompK36 gene from Z2110 and Z2554 is shown in Fig. 4. These mutations resulted in alteration of the ORF and disruption of the ompK36 gene. These results suggested that it was OmpK36 that was not expressed in *K. pneumoniae* Z2554 and Z2110.

**IEF analysis**

IEF analysis of the two isolates and the *E. coli* transconjugant of Z2110 demonstrated that there were two β-lactamases in *K. pneumoniae* Z2554 with pIs of 5.4 and 7.9, whilst *K. pneumoniae* Z2110 produced three β-lactamases with pIs of 5.4, 7.8 and 7.9. The *E. coli* transconjugant produced a single β-lactamase of pI 7.8 (data not shown).

**PCR and DNA sequence analysis**

To confirm the genotypes of the β-lactamases detected by IEF, PCR was conducted for plasmids extracted from *K. pneumoniae* Z2554 and Z2110 and the *E. coli* transconjugant. As a result, *bla*<sub>TEM</sub> and *bla*<sub>CTX-M-9</sub> group genes were detected in both clinical isolates, whilst the *bla*<sub>DHA</sub> gene was detected in *K. pneumoniae* Z2110 and the *E. coli* transconjugant. After comparing the DNA sequences with known genes in GenBank, we found that isolate Z2554 contained sequences encoding TEM-1 and CTX-M-14, whilst isolate Z2110 encoded an extra AmpC β-lactamase, DHA-1, and the *E. coli* transconjugant of Z2110 encoded a single DHA-1.

**SDS-PAGE and OMP-encoding gene analysis**

*K. pneumoniae* Z2554 and Z2110 showed various levels of resistance to carbapenems but did not produce any type of carbapenemase, indicating that other carbapenem resistance mechanisms, such as alteration in outer-membrane permeability or an efflux pump, might be involved. However, as the efflux mechanism is unlikely to be involved in the resistance of enterobacteria, we focused on an alteration in the OMPs. SDS-PAGE analysis of the OMPs was performed, and the result revealed loss of an OMP of ~39 kDa in both *K. pneumoniae* Z2554 and Z2110 compared with *K. pneumoniae* ATCC 13883 (Fig. 3). It was uncertain whether the 39 kDa OMP was OmpK36 or OmpK35, as in some strains OmpK36 migrates faster than OmpK35 (Hernández-Allés et al., 1999). The ompK35 gene was amplified, sequenced and aligned with that of *K. pneumoniae* KT755 (GenBank accession no. AJ011501) (Domènech-Sánchez et al., 2003), and point mutations were found at nt 420, 474, 537 and 786 on the nucleotide sequence in both *K. pneumoniae* Z2554 and Z2110, and an additional point mutation was present in Z2554 at nt 249. However, point mutations in the ompK35 gene were all silent and did not lead to amino acid sequence changes. Comparing the ompK36 gene of *K. pneumoniae* Z2554 and Z2110 with that of *K. pneumoniae* C3 (GenBank accession no. Z33506; Alberti et al., 1995), several small DNA fragment insertions and a number of point mutations were observed. The sequence of the ompK36 gene from Z2110 and Z2554 is shown in Fig. 4. These mutations resulted in alteration of the ORF and disruption of the ompK36 gene. These results suggested that it was OmpK36 that was not expressed in *K. pneumoniae* Z2554 and Z2110.
We realized that in strain Z2110, although OmpK36 was not expressed, there was an additional protein band of ~26 kDa seen by SDS-PAGE (Fig. 3). An early termination of translation might lead to a truncated protein of ~26 kDa – in other words, we suspected that the new ~26 kDa protein in Z2110 might be the truncated product of OmpK36. If that were the case, then it indicates that the truncated C-terminal portion of the OmpK36 protein plays a critical role in drug resistance. After SDS-PAGE and protein transfer, we analysed the N-terminal amino acid sequence of this protein. The 15 aa determined are shown in Fig. 5. Comparing the sequence of the determined N-terminal sequence with the putative sequence of the ompK36 gene, we were unable to find any similarity, suggesting that the newly discovered ~26 kDa protein from Z2110 was not a truncated OmpK36 protein. To understand the nature of this protein, we searched GenBank to look for potential homologous sequences. We found a hypothetical protein called KPN_03267 originating from K. pneumoniae subsp. pneumoniae MGH 78578 (GenBank accession no. YP_001336895), which showed 100% similarity to the 15 determined N-terminal amino acids. The sequence of this putative protein is shown in Fig. 5. This protein has 231 aa in total with an approximate molecular mass of 25.9 kDa, which is similar to the protein we observed in Z2110. However, transmembrane structure analysis using ConPre II software conducted for this protein revealed that there is no transmembrane topology in this sequence. Therefore, this protein is unable to fold into a transmembrane structure to form a channel, as does OmpK36. It is possible that the replacement of OmpK36 with a new protein of ~26 kDa resulted in the loss of the channel function, which eventually leads to resistance in the strain Z2110 in addition to the high-level expression of different types of β-lactamases. Currently, the contribution of this new protein to drug resistance in strain Z2110 is unclear and is worthy of further study.

K. pneumoniae contains two major porins, OmpK35 and OmpK36, which correspond to OmpF and OmpC, respectively, in E. coli, and OmpK37, which is not expressed or is expressed at very low levels under...
Laboratory conditions and is undetectable by SDS-PAGE (Doménech-Sánchez et al., 1999). Loss of either Ompk35 or OmpK36 in K. pneumoniae leads to reduced susceptibility to cefoxitin and cefotaxime (MIC 2–4 μg ml⁻¹), but maintains sensitivity to carbapenems (MIC 0.06–0.125 μg ml⁻¹). However, loss of both porins can result in reduced susceptibility to imipenem and meropenem (MIC 0.25–2 μg ml⁻¹) (Doménech-Sánchez et al., 1999). Loss of one of the porins combined with broad-spectrum β-lactamase and/or ESBL production in K. pneumoniae can cause resistance to cefoxitin and cephalosporins whilst maintaining susceptibility to imipenem (Martínez-Martínez et al., 1996; Rice et al., 1993), whilst combining with AmpC β-lactamase expression results in resistance or reduced susceptibility to carbapenems (Bradford et al., 1997; Cao et al., 2000; Lee et al., 2007b). This view is supported by the observations in K. pneumoniae Z2110 in the current study. Loss of both porins combined with broad-spectrum β-lactamase and/or ESBL production would result in moderate resistance or reduced susceptibility to imipenem and meropenem, as well as resistance to ertapenem (Crowley et al., 2002; Elliott et al., 2006; Jacoby et al., 2004). Lee et al. (2007a) reported that K. pneumoniae isolates Lkp11–13 that produced CTX-M-3 and SHV-5 but with OmpK35 deficiency and OmpK36 expression were susceptible to carbapenems. Genetically related K. pneumoniae Lkp14 producing an extra DHA-1 and failing to express OmpK36 showed resistance to ertapenem, and reduced susceptibility to imipenem and meropenem. The introduction of a blaDHA-1-carrying plasmid into Lkp11 only resulted in a significant increase in the MICs of cephapemicycins and β-lactamase inhibitors, and a slight increase in the MIC of ertapenem, but not in those of imipenem and meropenem. Mena et al. (2006) also reported that CTX-M-1-producing K. pneumoniae that expressed one of the two major porins, OmpK36, was susceptible to carbapenems, whereas isolates that did not express either of the porins were resistant to carbapenems. In this study, two clinical isolates of genetically unrelated, OmpK36-deficient, OmpK35-expressing K. pneumoniae were investigated. K. pneumoniae Z2110 producing TEM-1, CTX-M-14 and plasmid-mediated DHA-1 β-lactamase was resistant to ertapenem, and had reduced susceptibility to imipenem and meropenem as expected. K. pneumoniae Z2254 producing TEM-1 and CTX-M-14 but not DHA-1, however, showed a similar resistance phenotype for carbapenems. Thus, we believe that OmpK36 may play an important role in the resistance or reduced susceptibility to carbapenems in K. pneumoniae isolates producing AmpC, ESBL or broad-spectrum β-lactamase. To the best of our knowledge this is the first report of clinical isolates of K. pneumoniae with reduced carbapenem susceptibility that lacked OmpK36 but showed expression of OmpK35, and provides further evidence that OmpK36 may be more important than OmpK35 in resistance.

Jacoby et al. (2004) reported that most porin-deficient K. pneumoniae derivatives producing plasmid-mediated β-lactamases are resistant to ertapenem but have only slightly reduced susceptibility to imipenem and meropenem. A similar carbapenem efficacy for porin-deficient K. pneumoniae clinical isolates was observed in the current study. In summary, we have described two clinical isolates of K. pneumoniae deficient in OmpK36 with resistance to ertapenem and reduced susceptibility to imipenem and meropenem, and have provided a detailed molecular analysis of their antimicrobial drug resistance mechanisms. Expression of OmpK35 was not sufficient to recover resistance to ertapenem or the reduced susceptibility to imipenem and meropenem in these K. pneumoniae isolates, suggesting that OmpK36 plays an important role in the resistance or reduced susceptibility to carbapenems in K. pneumoniae isolates producing AmpC, ESBL or broad-spectrum β-lactamase.

**REFERENCES**


**Fig. 5.** The N-terminal amino acid sequence of the ~26 kDa protein from Z2110 compared with the putative amino acid sequence of hypothetical protein KPN_03267 (K. pneumoniae subsp. pneumoniae MGH 78578). The underlined sequence shown at the top is the N-terminal sequence of the protein from Z2110.


