Klebsiella pneumoniae type 3 fimbriae agglutinate yeast in a mannose-resistant manner

Steen G. Stahlhut, Carsten Struve and Karen A. Krogfelt

Department of Microbiological Surveillance and Research, Statens Serum Institut, 2300 Copenhagen S, Denmark

The ability of bacterial pathogens to express different fimbrial adhesins plays a significant role in virulence. Thus, specific detection of fimbrial expression is an important task in virulence characterization and epidemiological studies. Most clinical Klebsiella pneumoniae isolates express type 1 and type 3 fimbriae, which are characterized by mediation of mannose-sensitive agglutination of yeast cells and agglutination of tannic acid-treated ox red blood cells (RBCs), respectively. It has been observed that K. pneumoniae isolates agglutinate yeast cells and commercially available sheep RBCs in a mannose-resistant manner. Thus, this study was initiated to identify the adhesin involved. Screening of a mutant library surprisingly revealed that the mannose-resistant agglutination of yeast and sheep RBCs was mediated by type 3 fimbriae. Specific detection of type 1 fimbriae expression in K. pneumoniae was feasible only by the use of guinea pig RBCs. This was further verified by the use of isogenic fimbriae mutants and by cloning and expressing K. pneumoniae fimbrial gene clusters in Escherichia coli. Yeast agglutination assays are commonly used to detect type 1 fimbriae expression but should not be used for bacterial species able to express type 3 fimbriae. For these species, the use of guinea pig blood for specific type 1 fimbriae detection is essential. The use of commercially available sheep RBCs or yeast is an easy alternative to traditional methods to detect type 3 fimbriae expression. Easy and specific detection of expression of type 1 and type 3 fimbriae is essential in the continuous characterization of these important adhesive virulence factors present in members of the Enterobacteriaceae.

INTRODUCTION

Klebsiella pneumoniae is recognized as an important opportunistic pathogen and is a common cause of urinary tract infections, respiratory tract infections and septicaemia, especially in immunocompromised individuals (Podschun & Ullmann, 1998). In recent years, an emerging syndrome of community-acquired pyogenic liver abscess caused by highly virulent K. pneumoniae strains has occurred (Keynan et al., 2007; Ko et al., 2002; Lederman & Crum, 2005; Sobirk et al., 2010; Wang et al., 1998). These severe infections often occur in otherwise healthy individuals and are frequently complicated by devastating dissemination of the infection to the eyes and central nervous system. The rising incidence of serious K. pneumoniae infections stresses the need to elucidate the pathogenic mechanisms that are responsible for transforming this otherwise opportunistic pathogen into a highly virulent pathogen.

Adherence to tissue surfaces as well as artificial surfaces is an important first step for bacteria in the development of infections. Using PCR and anti-fimbrial serum, it has been established that >80 % of K. pneumoniae isolates are able to express the two fimbrial adhesins type 1 and type 3 fimbriae (Schurtz et al., 1994; Stahlhut et al., 2009; Tarkkanen et al., 1992). Type 1 fimbriae, originally described in the late 1950s, are present in the majority of enterobacterial species (Duguid et al., 1955). They belong to the chaperone/usher class fimbriae family and are encoded by fimABCDEFGHIK, with fimA being the major structural subunit whilst fimH encodes the adhesin subunit. FimH has been shown to mediate adhesion to mannose-containing structures present on host tissue surfaces and extracellular matrix (Klemm et al., 1990; Krogfelt et al., 1990; Madison et al., 1994). We recently established that type 1 fimbriae are essential for the ability of K. pneumoniae to cause urinary tract infections (Struve et al., 2008). Like type 1 fimbriae, type 3 fimbriae belong to the chaperone/usher class fimbriae. Type 3 fimbriae were first described in Klebsiella species exhibiting mannose-resistant haemagglutination of tannic acid-treated ox erythrocytes (Duguid, 1959; Thornley & Horne, 1962). In addition to Klebsiella species, type 3 fimbriae are common in Enterobacter, Serratia, Proteus and Providencia isolates (Clegg et al., 1994). The fimbriae are encoded by the mrkABCDF gene cluster, where the mrkD gene encodes the...
fimbrial adhesin (Allen et al., 1991; Jagnow & Clegg, 2003; Langstraat et al., 2001). In vitro studies have revealed that type 3 fimbriae mediate adhesion to different structures in human kidney and lung tissue and to epithelial cells from human urine sediments, as well as to endothelial and bladder epithelial cell lines; however, the identity of the receptor remains elusive (Hornick et al., 1992; Tarkkanen et al., 1990, 1992, 1997). Furthermore, type 3 fimbriae have been established to play an essential role in 

K. pneumoniae biofilm formation (Di Martino et al., 2003; Jagnow & Clegg, 2003; Langstraat et al., 2001; Schroll et al., 2010). Historically, type 3 fimbriae have not been associated with

Escherichia coli; however, recently two independent studies have reported type 3 fimbriae expression in 

E. coli strains (Burmølle et al., 2008; Ong et al., 2008). Intriguingly, in both studies, type 3 fimbriae were encoded by conjugative plasmids and were found to profoundly enhance the ability of 

E. coli to form biofilm. The ability of type 3 fimbriae to strongly promote biofilm formation in different bacterial species calls for further characterization of this important fimbrial type.

Fimbrial expression is traditionally detected by specific agglutination of red blood cells (RBCs) from different animal species and whether the agglutination reaction is inhibited by mannose. Thus, type 1 fimbriae are characterized by mediation of mannose-sensitive agglutination of guinea pig RBCs as well as yeast (Saccharomyces cerevisiae), whereas type 3 fimbriae are characterized by their ability to agglutinate tannic acid-treated, but not native, erythrocytes in a mannose-resistant manner (Allen et al., 1991; Korhonen et al., 1981; Krogfelt et al., 1990). In this study, we showed that type 3 fimbriae mediate mannose-resistant agglutination of commercially available sheep RBCs and yeast. This is a novel and easy method for detection of the important type 3 fimbriae.

### METHODS

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are listed in Table 1. Construction of a C3091 isogenic MrkD mutant is described below. The C3091 transposon mutant library has been described previously (Struve et al., 2003).

Bacteria were routinely cultured at 37 °C in Luria–Bertani (LB) broth or on LB agar plates containing the appropriate antibiotics at the following concentrations: 50 μg kanamycin ml⁻¹, 100 μg ampicillin ml⁻¹, 30 μg apramycin ml⁻¹ and 30 μg chloramphenicol ml⁻¹.

**Construction of isogenic fimbriae mutants.** The C3091 fimbriae mutants and their construction have been described previously in detail and the same procedure was used to construct the fimbriae mutants of strain 8223 (Struve et al., 2009).

The MrkD isogenic mutant was constructed as described previously (Struve et al., 2009). Briefly, the mrkD gene in C3091 was deleted by allelic exchange with a kanamycin resistance gene (kan)-encoding cassette flanked by regions homologous to the regions up- and downstream of the mrkD gene. The cassette was generated by a three-step PCR procedure. As the first step, the kan gene was amplified from pKD4 using the primer pair Kn1 and Kn2 (Datsenko & Wanner, 2000). Secondly, from C3091, chromosomal DNA flanking the mrkD gene was amplified by PCR using primer pairs UpmrkD-F (5'-GC-GGCGGCGTGGTGCTCTC-3') and UpmrkD-R (5'-GAAGACAGCTCCAGCTACACCTCATGTCGCTCGGTCAGAAAG-3'), and DwmrkD-F (5'-GGACCATTGGGTTCCATTTCCATTAGGAGCCCGCT-ACATCACCATTAC-3') and DwmrkD-R (5'-CAGATACCGGGCTTTTGTCGATTACC-3'), respectively. At their 5' ends, primers UpmrkD-R and DwmrkD-F contained 20 bp regions homologous to the extremities of the kan gene. In the third step, the flanking regions were added on each side of the kan gene by mixing 100 ng of each fragment, followed by PCR amplification using primer pair UpmrkD-F and DwmrkD-R. The PCR product was purified and electroporated into C3091 harbouring the thermosensitive plasmid pKOBEGApra encoding the λ Red recombinase. The C3091 mrkD mutant was selected by growth on LB plates containing kanamycin at 37 °C. Loss of the pKOBEGApra plasmid was verified by the inability of the mutant to grow on LB agar plates containing apramycin. Correct allelic exchange was verified by PCR analysis using combinations of

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3091</td>
<td>K. pneumoniae UTI isolate</td>
<td>Oelschlaeger &amp; Tall (1997)</td>
</tr>
<tr>
<td>C3091Amrk</td>
<td>Type 3 fimbriae cluster deleted in C3091</td>
<td>Struve et al. (2009)</td>
</tr>
<tr>
<td>C3091AmrkD</td>
<td>Adhesive subunit of type 3 fimbriae deleted in C3091</td>
<td>This study</td>
</tr>
<tr>
<td>C3091Amfim</td>
<td>Type 1 fimbriae cluster deleted in C3091</td>
<td>Struve et al. (2008)</td>
</tr>
<tr>
<td>C3091AmkDafim</td>
<td>Type 1 and 3 fimbriae cluster deleted in C3091</td>
<td>Struve et al. (2009)</td>
</tr>
<tr>
<td>8223</td>
<td>K. pneumoniae catheter-associated UTI isolate</td>
<td>This study</td>
</tr>
<tr>
<td>8223Amrk</td>
<td>Type 3 fimbriae cluster deleted in 8223</td>
<td>This study</td>
</tr>
<tr>
<td>8223Amfim</td>
<td>Type 1 and 3 fimbriae cluster deleted in 8223</td>
<td>This study</td>
</tr>
<tr>
<td>HB101</td>
<td>Non-fimbriated, non-capsulated E. coli K-12 laboratory strain</td>
<td>Purcell &amp; Clegg (1983)</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCAS625</td>
<td>pUC18_mrkABCDF, high-copy-number vector containing mrkABCDF</td>
<td>Struve et al. (2009)</td>
</tr>
<tr>
<td>pCAS624</td>
<td>pUC18_mrkABCDF, high-copy-number vector containing fimABCDFGHIK</td>
<td>Struve et al. (2008)</td>
</tr>
<tr>
<td>pmrkD</td>
<td>pGB17_mrkD, low-copy-number vector containing mrkD</td>
<td>This study; Sokurenko et al. (1994)</td>
</tr>
</tbody>
</table>
primers inside the *kan* gene, K1 and K2 (Datsenko & Wanner, 2000), and primers flanking the deleted *mrkD* gene, UpmrkA (5’-CCA-CGGCAAGGGAGATTCAGACT-3’) and DwmrkF (5’-TTACATT-TCCGCCATCTCCCATTA-3’).

**Agglutination assays on glass slides.** Bacterial culture was applied to a glass slide and mixed with a freshly prepared solution of 5% guinea pig erythrocytes (Statens Serum Institut), 1% yeast (Sigma) or 0.5% sheep RBCs (Sigma). The slide was gently rotated until agglutination was visible. Agglutination assays were performed in the presence or absence of 5% mannose.

**Haemagglutination screening of a transposon mutant library in microtitre plates.** Bacteria were grown overnight at 37 °C in LB media under shaking. An aliquot of culture (60 μl) was added to a U-bottomed 96-well microtitre plate (Nunc), followed by 60 μl PBS and 30 μl 0.5% solution of sheep RBCs. The plate was shaken at 600 r.p.m. for 10 min and placed at room temperature for 2 h, after which the haemagglutination reactions were read visually.

**Cloning and sequence analysis of attenuated mutants.** Cloning and sequencing were carried out as described previously (Struve et al., 2003). Briefly, chromosomal DNA was purified from attenuated mutants and digested with EcoRI or PstI. The DNA fragments were ligated into linearized pUC18 vector and the ligation reaction was used to transform *E. coli* DH5 to kanamycin resistance. Plasmids from *E. coli* were isolated isogenic type 3 fimbriae mutant of C3091 was used for transformation to non-fimbriated *E. coli* strain HB101 and the transformant was tested for agglutination. As expected, in contrast to the wild-type, the type 3 fimbriae mutant did not agglutinate sheep RBCs (C3091*mrk*; Table 2). However, when the type 3 fimbriae mutant was transformed with pCAS625, encoding the cloned type 3 fimbriae gene cluster from C3091 (Struve et al., 2009), the ability to agglutinate sheep RBCs in a mannose-resistant manner was restored [C3091*mrk*; Table 2]. To further verify these findings, pCAS625 was cloned into the non-fimbriated *E. coli* strain HB101 and the transformant was tested for agglutination. As expected, in contrast to the HB101 wild-type, HB101(pCAS625) strongly agglutinated sheep blood in a mannose-resistant manner (Table 2).

### RESULTS

**Screening of a mutant library for loss of ability to agglutinate sheep RBCs**

During investigation of a collection of eight *K. pneumoniae* isolates (Struve et al., 2009), we observed that all isolates markedly agglutinated yeast cells and commercially available sheep RBCs in a mannose-resistant manner. To identify the adhesin causing this phenotype, a library of 1440 transposon mutants of the clinical *K. pneumoniae* isolate C3091, which had been constructed previously (Struve et al., 2003), was screened for loss of ability to agglutinate sheep RBCs. The screening revealed four agglutination-negative mutants. To identify the disrupted genes of the agglutination mutants, the transposon insertion sites were identified by cloning and sequencing. The disrupted gene of one mutant was identified as *aroG*, encoding a 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase involved in the synthesis of aromatic amino acids. Surprisingly, in the remaining three mutants, the disrupted gene was identified as *mrkA*, encoding the major fimbrial subunit of type 3 fimbriae.

### Type 3 fimbriae mediate mannose-resistant agglutination of sheep RBCs

To verify that type 3 fimbriae mediate mannose-resistant agglutination of sheep RBCs, a previously well-characterized isogenic type 3 fimbriae mutant of C3091 was used (Schroll et al., 2010; Struve et al., 2009). In contrast to the wild-type, the type 3 fimbriae mutant did not agglutinate sheep RBCs (C3091*mrk*; Table 2). However, when the type 3 fimbriae mutant was transformed with pCAS625, encoding the cloned type 3 fimbriae gene cluster from C3091 (Struve et al., 2009), the ability to agglutinate sheep RBCs in a mannose-resistant manner was restored [C3091*mrk*; Table 2]. To further verify these findings, pCAS625 was cloned into the non-fimbriated *E. coli* strain HB101 and the transformant was tested for agglutination. As expected, in contrast to the HB101 wild-type, HB101(pCAS625) strongly agglutinated sheep blood in a mannose-resistant manner (Table 2).

### Type 3 fimbriae agglutinate yeast in a mannose-resistant manner

We speculated that type 3 fimbriae might also be responsible for the mannose-resistant agglutination of yeast by *K. pneumoniae*. Indeed, in addition to sheep RBCs, all four transposon mutants were unable to agglutinate yeast in the presence of mannose, indicating that the mannose-resistant agglutination of sheep RBCs and yeast was mediated by the same factor, type 3 fimbriae. Verifying that *K. pneumoniae*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sheep RBCs</th>
<th>Sheep RBCs + mannose</th>
<th>Yeast</th>
<th>Yeast + mannose</th>
<th>Guinea pig RBCs</th>
<th>Guinea pig RBCs + mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3091</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C3091<em>mrk</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C3091<em>mrkD</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C3091<em>fim</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C3091<em>mrkA</em>fim*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C3091<em>mrk(pCAS625)</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C3091<em>mrkD(pmrkD)</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HB101</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HB101(pCAS625)*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HB101(pCAS624)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
mannose-resistant yeast agglutination was not due to expression of type 1 fimbriae, an isogenic type 1 fimbriae mutant of C3091 was shown to still agglutinate yeast in a mannose-resistant manner (C3091Δfim; Table 2). In contrast, the type 3 fimbriae mutant of C3091 was unable to agglutinate yeast in the presence of mannose, and a C3091 type 1 and type 3 fimbriae double mutant did not agglutinate yeast at all (C3091ΔmrkDΔfim; Table 2). Furthermore, whereas E. coli HB101 was unable to agglutinate yeast, HB101 carrying the cloned type 3 fimbriae gene cluster from C3091 strongly agglutinated yeast in a mannose-resistant manner, confirming that type 3 fimbriae mediate mannose-resistant yeast agglutination [HB101(pCAS625); Table 2].

Agglutination of yeast and RBCs by type 3 fimbriae is not restricted to strain C3091

To verify that agglutination of yeast and sheep RBCs is generally mediated by type 3 fimbriae in different K. pneumoniae isolates, isogenic type 1 and type 3 fimbriae mutants and a type 1 and type 3 fimbriae double mutant of the catheter-associated urinary tract isolate 8223 were constructed. The agglutination phenotypes of the wild-type 8823 and its fimbriae mutants were identical to the phenotypes observed for strain C3091, indicating that type 3 fimbriae mediation of agglutination of yeast and sheep RBCs is a general trait among K. pneumoniae isolates.

Type 3 fimbriae mannose-resistant agglutination of sheep RBCs and yeast is mediated by the MrkD adhesin

To investigate whether the sheep RBC and yeast agglutination was mediated by the type 3 fimbriae adhesin MrkD or a structural component of the fimbriae, an isogenic mrkD mutant was constructed (C3091ΔmrkD). The MrkD mutant was unable to agglutinate sheep RBCs or yeast in the presence of mannose (Table 2). However, when the MrkD mutant was transformed with a plasmid encoding only the cloned mrkD gene from C3091 [C3091ΔmrkD(pmrkD); Table 2], the ability to agglutinate was restored. Thus, type 3 fimbriae mannose-resistant agglutination of sheep RBCs and yeast is mediated by the MrkD adhesin.

Guinea pig RBC agglutination is specifically mediated by type 1 fimbriae

Yeast agglutination is a common assay for detection of type 1 fimbriae expression. However, we have shown here that, in K. pneumoniae, yeast agglutination cannot be used for specific detection of type 1 fimbriae, as type 3 fimbriae also agglutinate yeast. Besides yeast, guinea pig RBCs are also known to be agglutinated by type 1 fimbriae in a mannose-sensitive manner. To ensure that type 3 fimbriae did not agglutinate guinea pig RBCs, K. pneumoniae fimbriae mutants and E. coli HB101 carrying the cloned type 1 (pCAS624) or type 3 (pCAS625) fimbriae gene cluster were tested (Table 2). We found that only type 1 fimbriae mediated guinea pig RBC agglutination. Thus, specific detection of type 1 fimbriae expression in K. pneumoniae is feasible by using guinea pig RBCs.

DISCUSSION

The expression of specific fimbriae in bacterial pathogens is generally detected by agglutination assays. Thus, the most common fimbrial type in members of the Enterobacteriaceae, type 1 fimbriae, is characterized by mediation of mannose-sensitive agglutination of guinea pig RBCs and yeast. As guinea pig RBCs may not be readily available, agglutination assays using yeast are a commonly used method for detection of type 1 fimbriae in many laboratories (Korhonen et al., 1981; Stahlhut et al., 2009). Whilst investigating a collection of K. pneumoniae isolates for type 1 fimbriae expression, we surprisingly observed that K. pneumoniae strongly agglutinated yeast, including in the presence of mannose, suggesting that K. pneumoniae may possess an unknown mannose-resistant adhesin. In addition to yeast, we also observed mannose-resistant agglutination of commercially available sheep RBCs by the K. pneumoniae isolates. To identify the adhesin, a transposon mutant library was constructed and the mutants were screened for their loss of ability to agglutinate sheep RBCs. Surprisingly, the screening revealed the agglutination to be mediated by type 3 fimbriae and aroG encoding a synthase involved in the synthesis of aromatic amino acids. In addition, the transposon mutants were also found to be unable to agglutinate yeast in the presence of mannose. It is unclear why the aroG gene influences agglutination of yeast and sheep RBCs. However, given the biosynthetic nature of the gene product, it could be speculated to affect the synthesis of type 3 fimbriae.

That type 3 fimbriae indeed mediated mannose-resistant agglutination of commercial sheep RBCs and yeast was verified by testing well-defined fimbriae mutants as well as by cloning the type 3 fimbriae gene cluster into the non-fimbriated E. coli strain HB101 (Table 2). Furthermore, the agglutination was shown to be mediated by the fimbrial adhesin MrkD, as agglutination was abolished when the mrkD gene was deleted. Agglutination ability was restored by complementation of the MrkD mutant with a plasmid containing the mrkD gene only, verifying that the abolition of agglutination was the result of deletion of the mrkD gene and not polar effects of the mutation, thus confirming the important role of the MrkD adhesin.

Type 3 fimbriae are characterized by their ability to agglutinate tannic acid-treated, but not native, RBCs in a mannose-resistant manner (Gerlach et al., 1989). The commercially available sheep RBCs used in this study were delivered as a glutaraldehyde-treated dry powder, and we speculate that either the drying or the glutaraldehyde treatment exposed type 3 fimbriae receptors on the RBCs that are not present on the surface of native RBCs. Indeed, we found that type 3 fimbriae did not agglutinate native sheep RBCs (results not shown).
We believe that our observation that type 3 fimbriae agglutinate yeast is of the utmost importance for the choice of methods for detection of fimbrial expression in *K. pneumoniae* and other species able to express type 3 fimbriae. Hence, in contrast to common practice, assays based on yeast agglutination should not be used for the detection of type 1 fimbriae expression in these species, as positive agglutination may also be due to the expression of type 3 fimbriae. As in other enterobacterial species, we found that *K. pneumoniae* guinea pig RBC agglutination was specifically mediated by type 1 fimbriae (Korhonen et al., 1981; Krogfelt et al., 1990). Thus, in *K. pneumoniae* and other species able to express type 3 fimbriae, guinea pig RBCs should be used for the detection of type 1 fimbrial expression. This includes *E. coli*, which may carry plasmids encoding type 3 fimbriae.

Traditionally, the detection of type 3 fimbriae is performed using tannic acid-treated ox erythrocytes (Duguid, 1959; Thornley & Horne, 1962). We suggest using commercially available dried sheep RBCs or yeast as an easy alternative to detect type 3 fimbriae expression, thereby circumventing the need to obtain fresh ox erythrocytes and tedious tannic acid treatment.

In conclusion, specific detection of fimbrial expression is an important task in virulence characterization and epidemiological studies. Yeast agglutination is frequently used for the detection of type 1 fimbriae expressed by most enterobacterial species. We found that the important opportunistic pathogen *K. pneumoniae* mediates mannose-resistant yeast agglutination by expression of type 3 fimbriae. Therefore, the use of guinea pig RBCs, and not yeast, to detect type 1 fimbriae in bacterial species able to express type 3 fimbriae is essential. For the detection of type 3 fimbriae, commercially available sheep RBCs or yeast are an easy alternative to traditional methods. The ability to easily detect and differentiate the expression of type 1 and type 3 fimbriae is central to the continuous characterization of these important virulence factors.

ACKNOWLEDGEMENTS

We thank Lukas Berchtold for help with the experimental work and Dennis Schrader Hansen, Department of Clinical Microbiology, Hillered Hospital, Denmark, for providing the *K. pneumoniae* 8223 strain. S. G. S. was partially supported by the Danish Research Agency grant 2101-06-0009.

REFERENCES


