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First evidence of polar flagella in *Klebsiella pneumoniae* isolated from a patient with neonatal sepsis

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The genus *Klebsiella* belongs to the family Enterobacteriaceae, and is currently considered to be non-motile and non-flagellated. In the present work, 25 *Klebsiella* strains isolated from nosocomial infections were assessed for motility under different growth conditions. One *Klebsiella* isolate, KpBUAP021, demonstrated a swim-like motility phenotype. The *K. pneumoniae* genotype was confirmed by 16S rRNA and *rpoB* gene sequence analysis. Multilocus sequence typing analysis also revealed that the KpBUAP021 strain places it in the ST345 sequence type, and belongs to the phylogenetic KpI group. Transmission electron microscopy and the Ryu staining technique revealed that KpBUAP021 expresses polar flagella. Finally, the presence of *fliC*, *fliA* and *flgH* genes in this *K. pneumoniae* strain was confirmed. This report presents the first evidence for flagella-mediated motility in a *K. pneumoniae* clinical isolate, and represents an important finding related to its evolution and pathogenic potential.

INTRODUCTION

The *Klebsiella* genus is frequently associated with urinary tract infections, sepsis and pneumonia in hospitalized patients (Pan *et al.*, 2008). In the USA and Europe, *Klebsiella pneumoniae* is responsible for 14–20 % of nosocomial infections, causing significant morbidity and mortality (Borer *et al.*, 2009; Ko *et al.*, 2002; Podschun & Ullmann, 1998). Among *K. pneumoniae*, virulence factors are the presence of siderophores, capsule, fimbrial adherence factors, including type 1, type 3 and KPF28 pili and the non-fimbrial adhesin CF29K (Alcantar-Curiel *et al.*, 2013; Di Martino *et al.*, 1995; Podschun & Ullmann, 1998).

For several pathogenic bacteria, motility is recognized as a major virulence factor because it facilitates colonization of target host tissues. It has been shown that *Listeria monocytogenes* and *Vibrio anguillarum* require flagella-mediated motility to invade host cells (O'Neil & Marquis, 2006; Ormonde *et al.*, 2000) and *Helicobacter pylori* uses flagella to colonize the stomach (Eaton *et al.*, 1996).

The flagellum is the main motility organelle present in bacteria and constitutes a complex multi-subunit machine. It is anchored to the membrane and emerges as one or more filaments (Liu & Ochman, 2007; McCarter, 2006; Thormann & Paulick, 2010). At least 50 genes are required for flagellar assembly (Macnab, 1992), where the *fliC* gene encodes for flagellin, the main flagellar structural protein. This gene is highly conserved in the 5′ and 3′ termini between Gram-

Abbreviations: BLAST, basic local alignment sequence tool; DIC, differential interference contrast; IBT, Institute of Biotechnology; JEM, Jeol transmission electron microscope; MEGA, Molecular Evolutionary Genetic Analysis; MIO, motility-indole-ornithine decarboxylase; MLST, multilocus sequence typing; NCBI, National Center for Biotechnology Information; PTA, phosphotungstic acid; TEM, transmission electron microscopy; TSA, tryptone soy agar; UPGMA, unweighted pair group method with arithmetic mean.

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negative members, making it a suitable marker for epidemiological and phylogenetic studies in many bacterial species (Winstanley & Morgan, 1997).

Several studies have revealed that some non-motile bacteria such as clinical isolates of enteroinvasive *Escherichia coli*, *Brucella melitensis, Shigella* and *Actinobacillus pleuropneumoniae* are indeed motile when grown under specific conditions, and this motility has been shown to be mediated by flagella (Andrade *et al.*, 2002; Fretin *et al.*, 2005; Giron, 1995; Negrete-Abascal *et al.*, 2003).

To date, *K. pneumoniae* has been considered a non-flagellated bacterium. Moreover, this phenotype is considered an important criterion to differentiate this genus from others within the Enterobacteriaceae family (Ewing, 1986). However, flagellum expression would provide a new virulence factor, increasing their mobility to efficiently colonize different anatomical niches. Furthermore, in 2013, a new *Klebsiella* species was identified and catalogued as *K. michiganensis,* and these organisms contain flagellar apparatus genes (Saha *et al.*, 2013). In the present study, we evaluated whether clinical isolates of *K. pneumoniae* can express flagella and are motile under different growing conditions. Our findings indicate that *K. pneumoniae* strain KpBUAP021, from a patient with neonatal sepsis, demonstrated motility and transmission electron microscopy (TEM) and Ryu analysis revealed the presence of polar flagella.

METHODS

Bacterial strains and growing conditions. Twenty-five *K. pneumoniae* strains were isolated from blood cultures of children with neonatal sepsis in the Hospital del Niño Poblano, Puebla, Mexico during 1998. Strains were identified by biochemical tests, including motility using the motility-indole-ornithine decarboxylase (MIO) medium test (Becton Dickinson Bioxon).

The strains were preserved in glycerol at −70 °C until use. In every culture and subculture, an aliquot was preserved at −70 °C and the motility phenotype was verified in each subculture. *K. pneumoniae* ATCC 700603, *E. coli* E2348/69 and *E. coli* AGT01 (E2348/69 *fliC*::*Cm* mutant) were used as controls for different assays. All the strains were grown in tryptone soy agar (TSA) (Becton Dickinson Bioxon) at 37 °C for 24 h under aerobic conditions and stored at −70 °C in TSA supplemented with 20 % glycerol.

Motility assay. Motility assays were performed using TSA plates and tubes with various agar concentrations (0.17, 0.20, 0.25, 0.3, 0.4, 0.5, 0.8 and 1.5 %) at different growth temperatures (25 , 30 , 37 and 42 °C), and different incubation times (5, 24, 36, 48 and 72 h). *K. pneumoniae* ATCC 700603 (non-motile) was used as a negative control and was grown under the same growing conditions as *K. pneumoniae* isolates. *E. coli* E2348/69 (motile) and AGT01 (*E. coli* non-motile) were used as positive and negative motility control strains, respectively. The *E. coli* strains were grown for 24 h at 37 °C. All the motility assays were performed three separate times, each time in triplicate.

***K. pneumoniae* (KpBUAP021) genotyping.** KpBUAP021 genotyping was performed by PCR amplification and sequencing of 16S rRNA and *rpoB* (β-subunit of RNA polymerase) genes, using the following oligonucleotides: 16SrRNAF, TTGGAGAGTTTGATCCTGGCTC; 16SrRNAR, ACGTCATCCCCACCTTCCTC; rpoBKpF, AACCAG TTCCGCGTTGGCCTGG and rpoBKpR, TTCCTGAATGTGGATAG TGG (Brisse & Verhoef, 2001; Mollet *et al.*, 1997). The sequenced fragments were analysed using the Basic Local Alignment Sequence Tool (BLAST) program on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST) and were aligned using ClustalW (http://www.ebi.ac.uk/ClustalW/index.html). The aligned sequences were analysed employing the Molecular Evolutionary Genetic Analysis (MEGA) 6 package, by the unweighted pair group method with arithmetic mean (UPGMA) method (500 bootstraps) (Tamura *et al.*, 2013).

Phylogenetic group identification. Phylogenetic group identification was determined by amplifying the *gyrA* gene with gyrAfiloKleb-F: CGCGTACTATACGCCATGAAGTA and gyrAfiloKleb-R: ACCG TTGATCACTTCGGTCAGG oligonucleotides. The amplicons were subjected to RFLP, using *Taq1* and *Hae*III restriction enzymes, and the restriction products were analysed on 2 % agarose gels (Brisse & Verhoef, 2001) and compared to the *gyrA* gene restriction pattern from the C1554 (KpI), C1555 (KpII) and C1556 (KpIII) strains (Ruiz *et al.*, 2010).

Multilocus sequence typing (MLST). MLST was performed as described by Diancourt *et al.* (2005) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). Chromosomal DNA was used to amplify and sequence seven housekeeping gene fragments from the *rpoB*, *gapA*, *mdh*, *pgi*, *phoe*, *infB* and *tonB* genes. The allelic profiles and sequence type were assigned using the *K. pneumoniae* MLST website (www.pasteur.fr/mlst).

Transmission electron microscopy. The KpBUAP021 strain was inoculated into the centre of a 0.25 % agar plate with a straight needle, and incubated under motility conditions. Bacteria were taken from the outside of the halo and placed in 500 µl of PBS, pH 7.4. The sample was diluted and placed on a nickel grid coated with carbon, and stained with 1 % phosphotungstic acid (PTA) at pH 6.8. The grids were observed using a Jeol transmission electron microscope (JEM 1400).

Ryu stain. The KpBUAP021 strain was stained using the Ryu stain protocol according to Kodaka *et al.* (1982). Briefly, the stain was prepared by mixing ten parts of a mordant solution (2 g tannic acid, 10 ml 5 % phenol, 10 ml saturated aluminium potassium sulfate 12-hydrate) and one part of stain solution (12 % crystal violet in ethanol). Bacteria were recovered gently from the outside of the motility halo formed in TSA plates and transferred to a water drop on a microscope slide. The preparations were air dried at room temperature, and stained for 10 min. Finally, samples were washed with tap water for 2 min. The preparations were observed under differential interference contrast (DIC) using a Nikon Eclipse Ti-E microscope. The images were captured using NIS elements software, version 4.20.

Detection of *fliC, fliA* and *flgH* genes. PCR amplification of different flagella genes and subsequent sequencing were performed using the following oligonucleotides: for *fliC*, D2 primer GCACAAGTCATTAG TACCAACAGCCTC and R2: GGCCTGCTGGATGATCTGCG; for *fliA*, FliaF CGTGGCGCTATGCGTGATGAACTT and FliaR: TGGCCTGGC TGTGTAACTGACTGA; and for *flgH,* flgHF: GCATACTTATGCCA TTTCCAGCTTGTTGG and fgHR: CGTTTCTTCCTTAACC TGTCGCCAATGT. These degenerate specific oligonucleotides were designed according to the nucleotide sequences reported in the GenBank database for the different genes of *E. coli* K12 (ID 949101), *Shigella flexneri* (ID 1078378) and *Salmonella enterica* subsp. *enterica* serovar Typhi (ID 1248507). The PCR products were purified using the Zymo Gel Extraction Kit, and sequenced at the Institute of Biotechnology(IBT), Cuernavaca, Morelos, Mexico.

RESULTS

Motility in a *K. pneumoniae* isolate

Twenty-five nosocomial isolates from paediatric patients were identified by biotypification as *K. pneumoniae*. Of these 25 strains, one strain (KpBUAP021) demonstrated motility when grown on MIO medium. In order to evaluate motility in all strains under different conditions, we included changes in temperature, incubation time and agar concentration. At 37 °C, the KpBUAP021 strain showed increased motility relative to the other clinical isolates using 0.25 % agar concentrations on plates and tubes. This strain demonstrated enhanced mobility at 36 h incubation time. The KpBUAP021 motility phenotype observed on the motility plates was identical to the swarming motility displayed by *E. coli* E2348/69. The KpBUAP021 strain showed a different growth pattern than that of the *K. pneumoniae* ATCC 700603 and *E. coli* AGT01 strains used as the negative motility controls (Fig. 1).

KpBUAP021 is a *K. pneumoniae* strain as revealed by genotyping

To confirm our isolate as *K. pneumoniae*, we performed a genotype analysis after amplifying and sequencing the 16S rRNA and *rpoB* genes. These genes have been used to study the phylogeny and taxonomy of *K. pneumoniae* strains (Brisse & Verhoef, 2001; Mollet *et al.*, 1997). The sequence of the 16S rRNA and *rpoB* genes from KpBUAP021 displayed 99 and 98 % similarity with *K. pneumoniae* M-X2C and *K. pneumoniae* NTUH-K2044, respectively.

Alignment of the 16S RNA and *rpoB* genes from the KpBUAP021 strain was used to construct a phylogenetic tree (MEGA 6 Package). For this tree, we included different *Klebsiella* species sequences from GeneBank, *Raoultella* and *Enterobacter* genera and sequences from *Staphylococcus aureus* as an out-group. As shown in Fig. 2, KpBUAP021 16S rRNA analysis demonstrated that the strain is part of a cluster and belongs to an ancestral node including *K. pneumoniae* subsp. pneumoniae. Similarly, the *rpoB* gene tree (Fig. 3) indicated a relationship with the *K. pneumoniae* subsp. pneumoniae group. Bootstrap tree topologies (Figs. 2 and 3) revealed few sequence differences between the 16S rRNA and *rpoB* genes compared to their homologues from *K. pneumoniae* subsp. pneumoniae. Moreover, KpBUAP021 is separated from the other *Klebsiella* species, *K. variicola* and *K. oxytoca*, and from some *Raoultella* and *Enterobacter* genera. The phylogenetic group of KpBUAP021 was determined by analysing the *gyrA* gene restriction pattern using C1554 (KpI), C1555 (KpII) and C1556 (KpIII) strains for comparison. Our results indicated that KpBUAP021 belongs to the Kpl phylogenetic group, in which three *K. pneumoniae* subspecies have been included. In addition, MLST analysis using seven housekeeping genes (*gapA-2, infB-1, mdh-5, pgi-2, phoE-4, rpoB-4* and *tonB-12*) indicated that KpBUAP021 belongs to the ST345 sequence type of *K. pneumoniae* (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

Observation of polar flagella in KpBUAP021

TEM was employed to investigate the presence of flagella in KpBUAP021. The results revealed that KpBUAP021 expresses a single polar flagellum with the characteristic undulating morphology (Fig. 4a, b). The average length of the flagellum was 10 µm, with an average width of 23 nm.

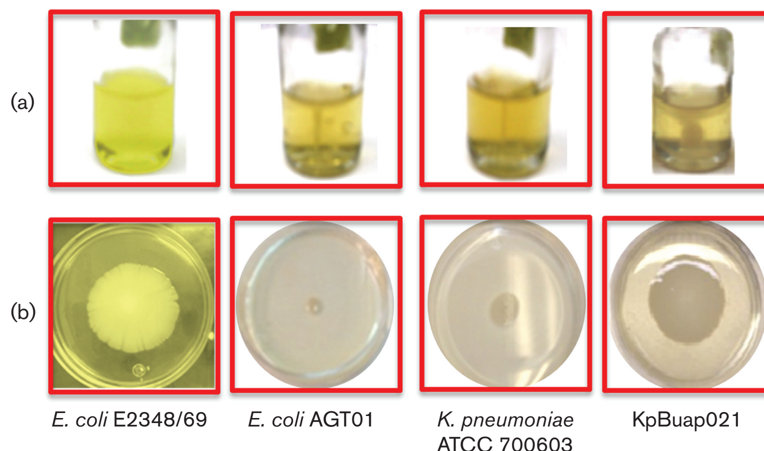

Fig. 1. *K. pneumoniae* motility assays. Control strains: *K. pneumoniae* ATCC 700603 (non-motile), *E. coli* AGT01 (non-motile), *E. coli* E2348/69 (motile) and *K. pneumoniae* (KpBUAP021) inoculated in: (a) tubes containing TSA and 0.25 % agar or (b) on TSA plates and 0.25 % agar. Bacterial cultures were maintained at 37 °C, for 24 and 36 h incubation for *E. coli* and *K. pneumoniae*, respectively. Representative images from three independent experiments are shown in which the KpBUAP021 strain demonstrated swim-like motility compared to *E. coli* E2348/69.

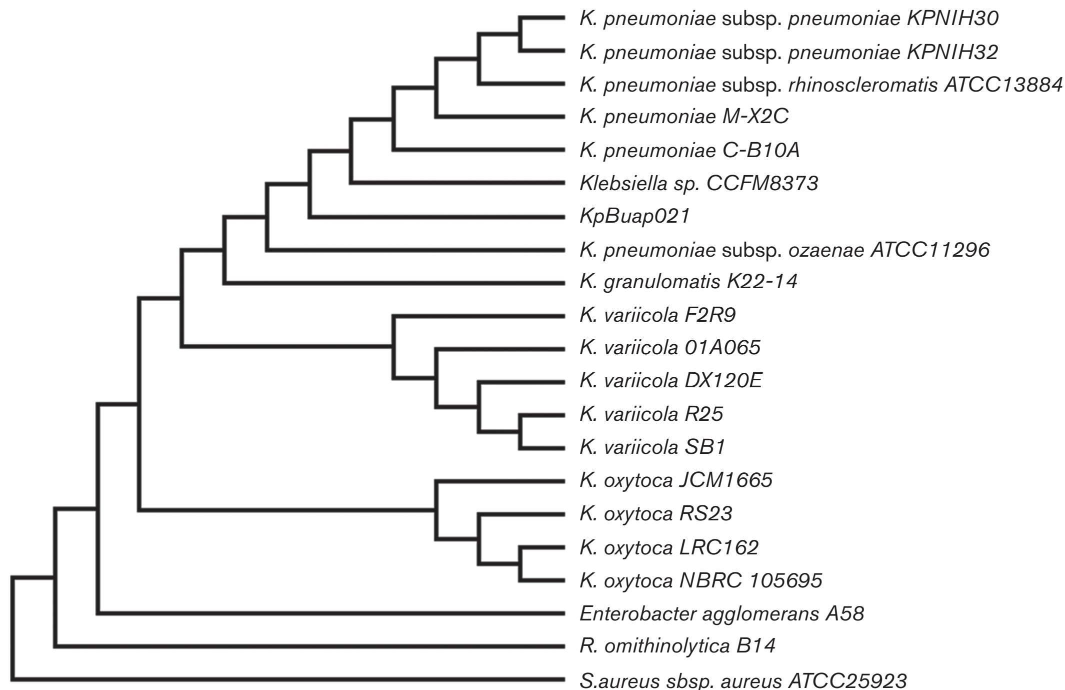

Fig. 2. Neighbour-joining analysis derived from the 16S rRNA partial gene sequence of KpBUAP021 and *K. pneumoniae* subsp. CCFM8373 (KJ803930.1), CB10A (KJ806502.1), M-X2C (KJ06466.1), 32192 (CP010361.1), ATCC1388 (NR_114507.1), KPNIH30 (CP009872.1), KPNIH32 (CP009775.1), ATCC11296 (NR_041750.1); *K. variicola* strains: 01A065 (HG933294.1), DX120E (CP009274.1), R25 (KM019906.1), 01A065 SB1 (HG933294.1), F2R9 (NR_025635.1); *K. oxytoca*: strains JCM 1665 (AB626120.1), RS23 (HF678365.1), LRC162 (JF772070.1), NBRC105695 (AB682268.1); *K. granulomatis*: K22-14 (EU333881.1); *Raoultella ornithinolytica*: B14 (JX680977) and *Enterobacter agglomerans*: A58 (AF130928.2). *S. aureus* subsp. aureus ATCC25923 (CP009361.1) was used as an out-group. Evolutionary distances were computed using the Poisson correction method. Branch support values (500 bootstraps) for nodes were realized. Brackets show GenBank accession numbers.

As demonstrated in Fig. 5(a), the Ryu staining technique also revealed the presence of a single polar flagellum in KpBUAP021. Although the number of flagellated bacteria in the preparation was low, the flagella produced by KpBUAP021 were detected by conventional optical microscopy. Fig. 5(b, c) shows the positive control *E. coli* E2348/69 (motile) and the negative control *E. coli* AGT01 (non-motile), respectively.

KpBUAP021 strain possesses the *fliC, fliA* and *flgH* genes

We next used PCR to determine whether the KpBUAP021 strain possesses the genetic elements required to express flagella. Using specific primers we identified the flagellin gene *fliC*, an intermediate gene, *flgH* and a regulatory gene, *fliA*. Our PCR amplification of *fliC* resulted in a weak band of 1.4 kbp (Fig. 6a) which was partially sequenced at the 5′ and 3′ ends, showing 98 % identity in comparison to the *fliC* gene from *S. flexneri*, and 91 % identity relative to *fliC* from *E. coli* O157:H7. Surprisingly, the PCR reaction amplified a second product of ~900 bp that showed no homology with *fliC*, but presented 74 % homology with a transcription factor of *K. pneumoniae* MGH 78578. The sequence of the 620 bp *fliA* PCR product from KpBUAP021 showed 99 % identity relative to the *E. coli* K12 *fliA* gene (Fig. 6b), and the 679 bp PCR product showed 99 % identity in comparison to the *flgH* gene from *E. coli* K12 and *S. flexneri* 2a str 301 (Fig. 6c).

DISCUSSION

Although *K. pneumoniae* is recognized as an important Gram-negative opportunistic pathogen, and some of its virulence factors and their role in pathogenesis are well known, the organism may express other, as yet undiscovered, virulence factors that could be involved in its ability to colonize different host anatomical sites. *K. pneumoniae* infections are related to the nature and number of virulence factors they express and, importantly, to host immune response (Yu *et al.*, 2007). In this study, we demonstrated that one *K. pneumoniae* strain (KpBUAP021) isolated from a case of neonatal sepsis displayed motility

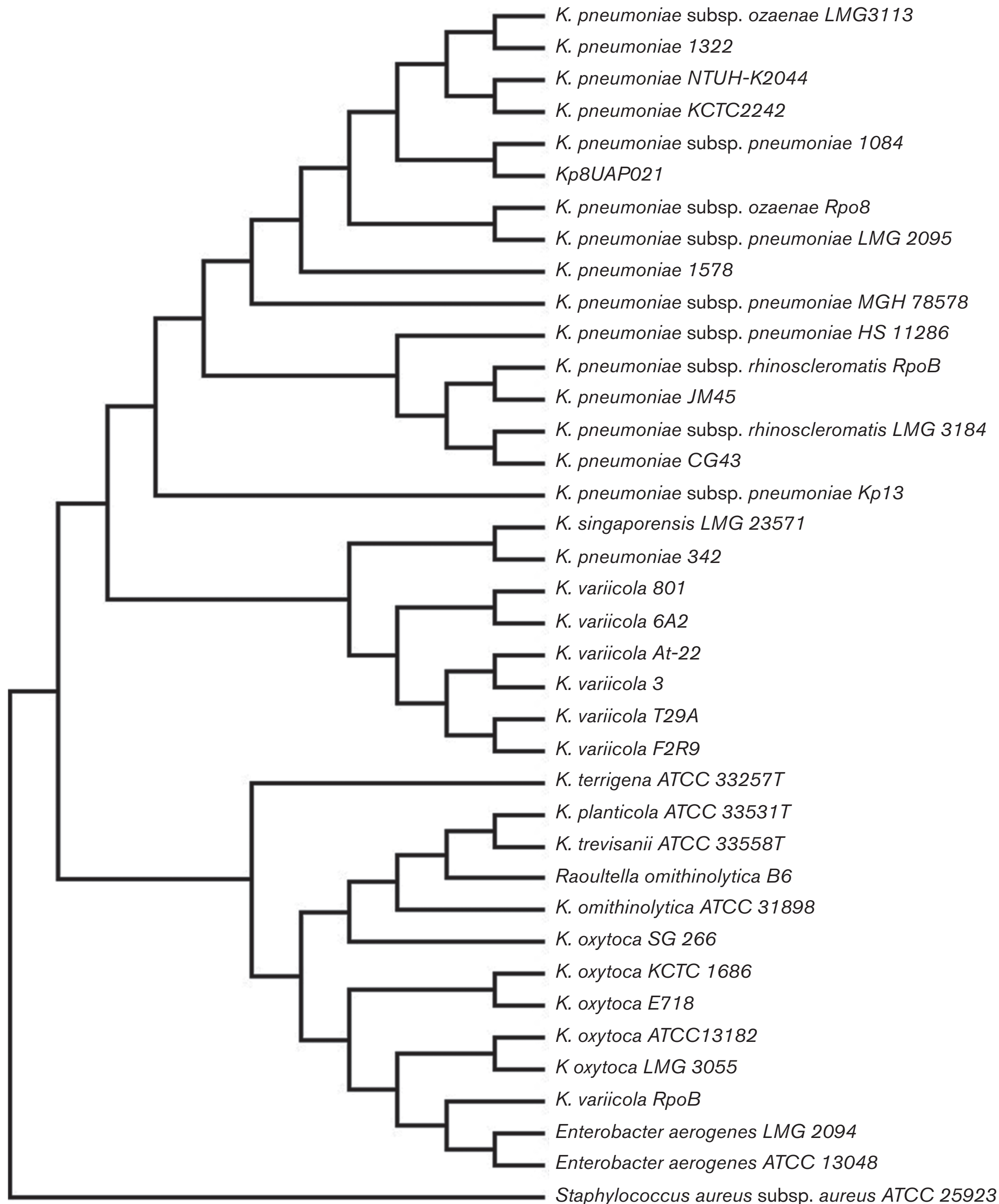

Fig. 3. Neighbour-joining analysis derived from the *rpoB* partial gene sequence obtained from KpBUAP021 and *K. pneumoniae* subsp. NTUH-K2044 (NC_012731.1), KCTC 2242 (NC_017540.1), CG43 (NC_022566.1), subsp. rhinoscleromatis: RpoB (AF129446.1), subsp. ozaenae: RpoB (AF129445.1), 1322 (AY367359.2), LMG2095 (JX425351.1), LMG3184 (JX425352.1), LMG3113 (JX425350.1), Kp13 (JN377745.1), ATCC13883 (EU010093.1), 1578 (AY367360.2), F11AB (AY016748.1), 342 (NC_011283.1), 1084 (NC_018522.1), HS11286 (NC_016845.1), JM45 (NC_022082.1). *K. variicola* strains: F2R9 (AY367356.2), At-22 (NC_013850.1), RpoB (EF416302.1), T29A (AY367353.2), 801 (AY367354.2), 6A2 (AY367355.2), 3 (AY367358.2). *K. oxytoca* strains: E718 (NC_018106.1), KCTC1686 (NC_016612.1), E718 (NC_018106.1), SG266 (AF491282.1), ATCC13182 (AY367363.2), LMG3055 (JX425349.1), ATCC13182 (EU010109.1). *K. planticola*: ATCC33531 (AF129449.1); *K. trevisanii*: ATCC 33558T (AF129450.1). *K. terrigena*: ATCC33257T (AF129448.1). *Raoultella ornithinolytica* strains: B6 (NC_021066.1), ATCC 31898 (AF129447.1). *Enterobacter aerogenes* strains: LMG2094 (DQ836222.1), ATCC13048 (KF516433.1). *S. aureus* subsp. aureus ATCC25923 (CP009361.1) was used as an out-group. Evolutionary distances were computed using the Poisson correction method. Branch support values (500 bootstraps) for nodes were realized. Brackets show GenBank accession numbers.

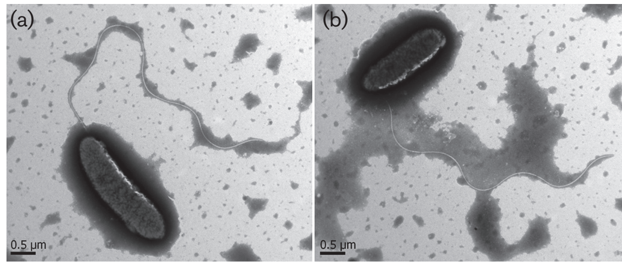

Fig. 4. TEM images of flagella expressed by *K. pneumoniae* KpBUAP021. (a) and (b) showing one polar flagellum with the characteristic wave-like morphology. Photographs are representative of two independent experiments in which several fields were analysed.

in vitro, and hence we decided to determine the genetic and phylogenetic relation of this strain. The genus classification of *Klebsiella* has recently been of interest in research, and a number of studies have revealed the high taxonomic complexity of these organisms (Brisse & Verhoef, 2001; Brisse *et al.*, 2014; Jonas *et al.*, 2004). Various studies have reported that a considerable proportion of isolates previously classified as *K. pneumoniae* could in fact be other bacteria, including *Raoultella planticola*, *R. terrígena*, *K. variicola* or *Enterobacter aerogenes* (Alves *et al.*, 2006; Brisse *et al.*, 2006). Taking this into consideration, we verified that our isolate represented a true *K. pneumoniae* strain.

Neighbour-joining analysis using the *rpoB* and 16S rRNA sequences revealed that KpBUAP021 is clustered with different *K. pneumoniae* subsp. pneumoniae. The phylogenetic analysis of *rpoB* confirmed that KpBUAP021 is a *pneumoniae* species, but this strain could not be separated completely from other subspecies of *K. pneumoniae* such as *Klebsiella* sp. CCFM8373, a faecal strain obtained from healthy donors that was co-isolated along with *K. pneumoniae* strains (Mao *et al.*, 2014). We also showed a separation with other species of *Klebsiella*, and with *Raoultella* and *Enterobacter* genera, in both phylogenetic analyses.

Furthermore, based on its *gyrA* gene restriction pattern, we were able to classify KpBUAP021 within the KpI phylogenetic group according to the Brisse *et al.* classification scheme (Brisse *et al.*, 2004; Brisse & Duijkeren, 2005). This result is of importance because it is known that determination of phylogenetic relationships between strains can reveal insights into the epidemiological pattern of bacterial species, as well as the evolution of their pathogenic potential. These phylogenetic relationships provide a framework with which to study the distribution of phenotypic properties implicated in bacterial epidemiology and their virulence (Brisse *et al.*, 2004). Moreover, phylogenetic analysis of housekeeping genes showed that the KpBUAP021 strain falls within the ST345 MLST sequence type.

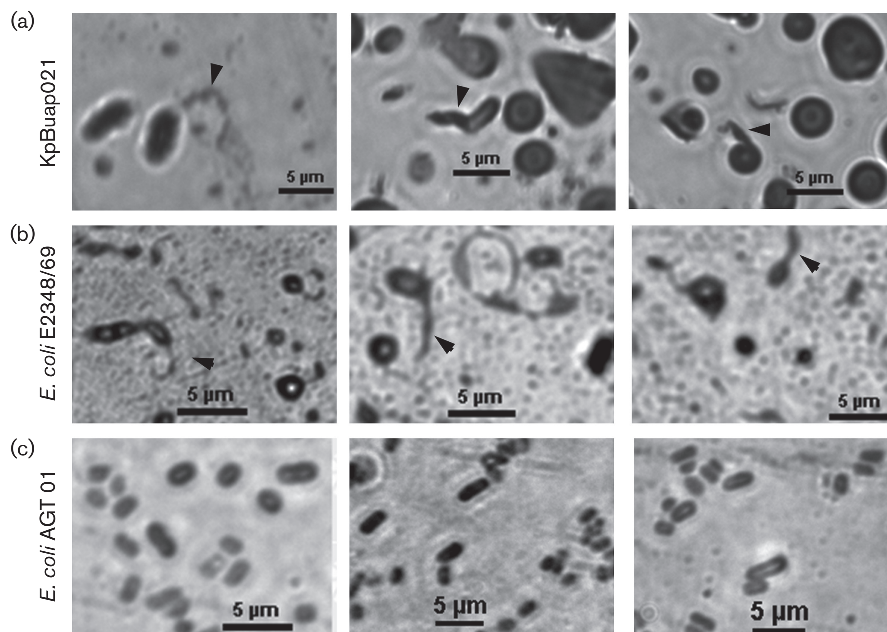

Fig. 5. Flagella detection in *K. pneumoniae* KpBUAP021 with Ryu stain. Bacteria were grown under motility-inducing conditions, stained with Ryu stain and visualized using light microscopy at ×100 magnification. Photographs are representative of three independent experiments in which several fields were analysed. (a) KpBUAP021 (motile) with polar flagellum. (b) Flagellum in *E. coli* E2348/69 (motile control strain) is demonstrated. (c) *E. coli* AGT 01 (non-motile control strain), had no flagella. The images were captured with Nis-Elements software. The flagella are indicated by arrowheads.

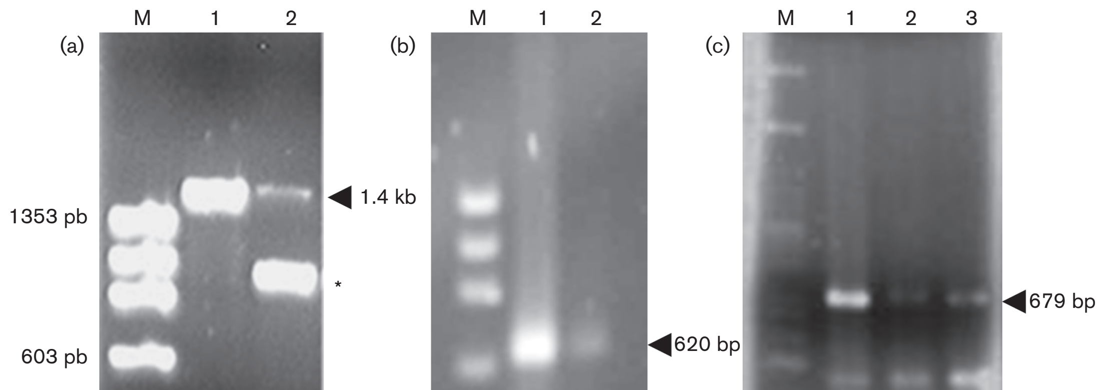

Fig. 6. PCR amplification of *fli*C, *fli*A and *flg*H genes from *K. pneumoniae* KpBUAP021. (a) *fli*C gene. Lane M, marker ϕ174; lane 1, *E. coli* E2348/69 (motile); lane 2, KpBUAP021. (b) *fli*A gene. Lane M, marker ϕ174; lane 1, *E. coli* E2348/69 (motile); lane 2, KpBUAP021. (c) *flg*H gene. Lane M, marker GeneRuler 100 bp Plus DNA Ladder; lane 1, *E. coli* E2348/69 (motile); lanes 2 and 3, KpBUAP021. PCR products corresponding to the genes amplified are indicated with an arrowhead. The asterisk indicates an unspecific PCR product.

During infections the expression of many bacterial virulence factors was specifically induced within the host–one example being flagella (Lai *et al.*, 2001). Previous reports indicate that flagella-mediated swarming motility and biofilm formation play an important role in the pathogenesis of some micro-organisms, especially in host colonization (Cong *et al.*, 2011; Harshey, 2003; Kearns, 2010). Several studies have reported that the percentage of agar is an essential stimulus for swimming motility after cell differentiation. Some bacteria can display swimming motility at agar concentrations as low as 0.3 % or less (Andrade *et al.*, 2002; Giron, 1995; Tercero-Alburo *et al.*, 2014). In the present study, the KpBUAP021 strain demonstrated swimming motility in 0.25 % agar notwithstanding that *K. pneumoniae* has been reported as a non-motile bacterium. Similar results have been reported for *E. coli* and *Shigella* (Andrade *et al.*, 2002; Giron, 1995) were flagellar expression has been linked to specific biological micro-environments or different growing conditions *in vitro* (Andrade *et al.*, 2002; Giron, 1995; Hughes *et al.*, 1993; Schmitt *et al.*, 1994). In our study, only one of 25 *K. pneumoniae* isolates showed motility as revealed by TEM analysis and Ryu staining (Figs. 4 and 5). In addition, flagellar size and morphology in KpBUAP021 are similar to those of flagella in many bacterial genera (Cruz-Cordova *et al.*, 2012; De Oliveira-Garcia *et al.*, 2002; Martinez *et al.*, 2009; Negrete-Abascal *et al.*, 2003).

Our results demonstrate that KpBUAP021 possesses genes that could be involved in the regulation (*fliA*) and composition (*flgH* and *fliC*) of the flagellum apparatus. Identification of the *fliA* gene in KpBUAP021 indicates that the regulatory machinery for expression of late genes in this strain is present. Other structural genes required for the production of flagella, but not universally distributed across flagellated species, include *flgH* (encoding the ring L). The *flgH* gene is also present in the motile KpBUAP021 strain. Additional genes need to be investigated to completely catalogue the structural set of genes [e.g. *flgF* (ring MS) and *flK* (rig P)] in KpBUAP021.

The N- and C-terminal portions of flagellin are important for flagella formation and are the most conserved regions. On the other hand, in enterobacterial flagellins, the middle region presents variability among strains (Andrade *et al.*, 2002; McQuiston *et al.*, 2004; Reid *et al.*, 1999). The detection of the *fliC* gene encoding flagellin (Doll & Frankel, 1993) and sequencing of the N- and C-terminal portions of the *fliC* gene in KpBUAP021 showed a similarity >90 % compared to the *fliC* genes from *E. coli* and *Shigella*.

Finally, our results indicate that *K. pneumoniae* is capable of expressing flagella under certain conditions, thereby enabling bacterial motility. This could be an important feature in *K. pneumoniae* pathogenicity by allowing it to reach and colonize various anatomical niches within the host. The expression of flagella could also be linked to other pathogenic mechanisms, such as biofilm formation. Further studies are required to determine the exact role of flagella in these bacteria and how they contribute to their pathogenic potential.

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