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Host and pathogen factors in Klebsiella pneumoniae upper urinary tract infections in renal transplant patients

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Abstract:	Purpose: To analyze the role of virulence factors (VFs) and host in Klebsiella pneumoniae upper urinary tract infections (UTIs) in renal transplant (RTx) recipients. Methodology: Clinical and demographic data were registered prospectively. Phylogenetic background of K.pneumoniae isolates was analyzed by PCR Melting Profiles (MP) and the following VFs genes: fimH-1, uge, kpn, ycfM, mrkD, rmpA, magA, hlyA, cnf-1, irp-1, irp-2, fyuA, entB, iutA, iroN by PCR. Results: We studied urine cultures and clinical data from 61 episodes of K. pneumoniae UTI in 54 RTx recipients. There were 32 cases of AB (53%), 10 cases of lower UTI (16%), 19 cases of AGPN (31%), including 6 cases of bacteraemia. 74% of strains were ESBL+, and there were two carbapenemase producing strains. PCR MP typing showed a diverse population with 52 different genetic profiles of K. pneumoniae. Analysis of the DNA profiles indicated 45 unrelated, unique genotypes and 7 related (16 isolates from 15 patients) genotypes. Urine flow impairment emerged as an independent predictor of K.pneumoniae upper UTIs (OR 14.28, CI 2.7–75.56, p 0.002), while we did not find any association between VFs profile and developing upper UTIs. The prevalence of the uge gene was lower in RTx patients on everolimus when compared to isolates from patients not receiving mTOR inhibitors (33.3% vs 82.8% p<0.05). Conclusions: K.pneumoniae upper UTI may be a marker of urine flow impairment. Bacterial virulence factors could not discriminate between upper and lower UTIs. However, immunosuppression may influence the selection of particular VFs.
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Host and pathogen factors in *Klebsiella pneumoniae* upper urinary tract infections in renal transplant patients.

Running title: *Klebsiella pneumoniae* upper UTIs in RTx patients

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Key words: *Klebsiella pneumoniae*, urinary tract infection, virulence factors, host factors, renal transplant

Abbreviations: AB, asymptomatic bacteriuria; AGPN acute graft pyelonephritis; AR, acute rejection; ATG, antithymocyte globulin; ATN, acute tubular necrosis; CCI, Charlson Comorbidity Index; CFU, colony-forming unit; CI, confidence interval; CLSI, Clinical & Laboratory Standards Institute; CMV, cytomegalovirus; CPD, chronic peritoneal dialysis; CsA, cyclosporine; DGF, delayed graft function; ESBL, extended-spectrum beta-lactamases; ESRD, end-stage renal disease; HMV, hypermucoviscosity phenotype; MHD, maintenance haemodialysis; MMF, mycophenolate mofetil; MPS, mycophenolate sodium; mTOR, mammalian target of rapamycin; OR, odds ratio; PCR MP, PCR melting profile; RTx, renal transplant; tac, tacrolimus; UPGMA, unweighted pair-group method with arithmetic averages; UTI, urinary tract infection; VF, virulence factor; VUR, vesico-ureteral reflux

Abstract:

Purpose: To analyze the role of virulence factors (VFs) and host in *Klebsiella pneumoniae* upper urinary tract infections (UTIs) in renal transplant (RTx) recipients.

Methodology: clinical and demographic data were registered prospectively. Phylogenetic background of *K.pneumoniae* isolates was analyzed by PCR Melting Profiles (MP) and the following VFs genes: *fimH*-1, *uge*, *kpn*, *ycf*M, *mrk*D, *rmp*A, *mag*A, *hly*A, *cnf*-1, *irp-1, irp-2, fyuA, entB, iutA, iroN* by PCR.

Results: We studied urine cultures and clinical data from 61 episodes of *K. pneumoniae* UTI in 54 RTx recipients. There were 32 cases of AB (53%), 10 cases of lower UTI (16%), 19 cases of AGPN (31%), including 6 cases of bacteraemia. 74% of strains were ESBL+, and there were two carbapenemase producing strains. PCR MP typing showed a diverse population with 52 different genetic profiles of *K. pneumoniae*. Analysis of the DNA profiles indicated 45 unrelated, unique genotypes and 7 related (16 isolates from 15 patients) genotypes. Urine flow impairment emerged as an independent predictor of *K.pneumoniae* upper UTIs (OR 14.28, CI 2.7–75.56, p 0.002), while we did not find any association between VFs profile and developing upper UTIs. The prevalence of the *u*ge gene was lower in RTx patients on everolimus when compared to isolates from patients not receiving mTOR inhibitors (33.3% vs 82.8% $p<0.05$).

Conclusions: *K.pneumoniae* upper UTI may be a marker of urine flow impairment. Bacterial virulence factors could not discriminate between upper and lower UTIs. However, immunosuppression may influence the selection of particular VFs.

Introduction:

Urinary tract infections (UTIs) are a major cause of morbidity in renal transplant recipients [1]. Clinical manifestation of UTIs ranges from asymptomatic bacteriuria (AB) to urosepsis. Acute graft pyelonephritis (AGPN), including urinary-source bacteraemia episodes, may adversely affect both long-term graft, and RTx recipients, outcomes [2,3].

UTIs are primarily caused by *Enterobacteriaceae* including *Escherichia coli* and *Klebsiella pneumoniae.* Because of the common multidrug resistance of *Klebsiella pneumoniae,* infections caused by this pathogen are raising concern. Although *K. pneumoniae* was reported as the most common pathogen in recurrent UTIs during the first year after RTx [4], there are very few detailed reports on infections with this bacterium in the solid organ transplantation recipients [5]. In the RTx recipients, AB is the main manifestation of *K. pneumoniae* UTI, while ESBL+ strains are most commonly isolated in cases of AGPN and urosepsis [6]. Why UTI progresses to AGPN or urosepsis in some instances, but not in others, needs to be elucidated.

In our previously published preliminary analysis we failed to identify any evident host-related risk factors for the development of upper *K. pneumoniae* infections [6]. Therefore the aim of the current study was to evaluate both host and bacterial risk factors for the development of *K. pneumoniae* AGPN including urosepsis. The analysis of phenotype, virulence genes together with host factors potentially relevant to the development of *K. pneumoniae* upper UTIs, may provide some indications as to how to prevent life-threatening bloodstream infections.

Patients

Study design, data collection, and definitions:

We performed a prospective cohort study of patients who presented with *K. pneumoniae* bacteriuria at the time of admission, or developed it subsequently. Urine cultures were taken at the discretion of the attending physician. We analyzed urine cultures of RTx patients with reference to clinical data: etiology of end-stage renal disease (ESRD), age, gender, comorbidity (Charlson Comorbidity Index CCI), recurrent UTIs before RTx, dialysis modality, pre-transplant dialysis time, episodes of acute

rejection (AR), acute tubular necrosis (ATN), delayed graft function (DGF), use of a double-J ureteral stent, type of immunosuppression, induction therapy with monoclonal (basiliximab) and polyclonal antibodies (antithymocyte globulin ATG), cytomegalovirus (CMV) infections, and the presence of vesico-ureteral reflux (VUR) or strictures at the uretero-vesical junction, and lower urinary tract malformation as underlying causes of ESRD.

Immunosuppressive protocols

All patients were initially maintained on a triple or quadruple regimen, including induction with monoclonal (basiliximab) and polyclonal antibodies (ATG) in some patients. Patients received tacrolimus (tac) + mycophenolate mofetil (MMF)/sodium (MPS) + glucocorticosteroids or cyclosporine (CsA) + MMF/sodium + glucocorticosteroids or CsA + everolimus + glucocorticosteroids.

Prophylaxis for infections

All patients were administered ceftriaxone perioperatively, usually for 4 days. This antibiotic was recommended by the hospital's epidemiologist, as it takes account of the current antibiotic resistance of Gram negative strains. When donor's cultures returned positive, the antibiotic was chosen based on susceptibility profiles. All patients received 480mg of trimetoprim/sulfamethoxazole daily for 6 months.

Definitions of UTIs

We divided all UTIs into the four following types: (1) AB, (2) lower UTI, (3) upper UTI (AGPN), (4) urosepsis. The definitions were adopted from our earlier publications [1,6].

Control group

The control group consisted of strains isolated from cases of UTIs in non-RTx patients admitted to the Department of Nephrology, Transplantology and Internal Medicine. UTI was either the reason for admission or complicated the in-hospital stay.

Methods

Bacterial isolates and phenotypic methods

K. pneumoniae isolates were collected from urine samples of eligible RTx recipients. All *K. pneumoniae* isolates from non-RTx patients hospitalized in the Department of Nephrology, Transplantology and Internal Medicine in the same period of time as RTx patients, were used as controls. The ATCC reference strain (700603™) and the *K. pneumoniae* carbapenemase (KPC) clinical strain from another hospital were also used for genotyping.

Identification and antibiotic susceptibility was determined using the Vitek2 (bioMerieux) system. Susceptibility assays were interpreted according to CLSI guidelines (CLSI, 2011). ESBL production was confirmed by E-test ESBL strips (bioMerieux, Hazelwood, Mo., USA) and stored in -80°C until analysis. Carbapenemaseproduction was confirmed with β CARBA test (Bio-Rad, USA).

DNA isolation

Bacterial DNA was extracted from a single colony on an LA agar plate using the Genomic DNA Kit (Bioline, A Meridian Life Science Company). The DNA concentrations were measured using NanoDrop ND-100 (Thermo Fisher Scientific, Wilmington, USA) and were at a level of 10-40 ng/µl. For detection of the plasmid-born *rmp*A gene, total DNA were isolated by the standard alkaline lysis and ethanol precipitation.

PCR detection of virulence-associated genes

We used PCR to detect virulence genes encoding adhesins (*fimH-1, mrkD, kpn, ycfM*), siderophores (*irp-1, irp-2, fyuA, entB, iutA, iroN)*, protectines or invasins (*rmpA, magA*), toxins (*hlyA, cnf-1*), and the *uge* gene. The two multiplex PCRs were used for the detection of 6 virulence genes (system I: *uge, fimH-1, irp-2;* system II*: kpn, mrkD, ycfM)* as a home-made test. Other virulence factors were detected by simplex PCR. Brief characteristics of the virulence factors are provided in Table 1.

All PCR reactions were performed in 25 µl final volume containing 0.5µl of the template DNA, 2.5µl of 10× PCR buffer *Taq* with $(NH_4)_2SO_4$ and 0.6 µl Taq DNA polymerase (2U/µl) (BLIRT S.A. DNA Gdańsk, POLAND), 0.2mM of each dNTP, 2mM of 50mM $MgCl_2$, 0.4µM each of the primers.

The cycling protocol for the multiplex PCRs included: 94°C for 2min, 94°C for 30sec, 61.4°C and 52.8 °C respectively for multiplex I and II, for 30sec and 45sec at 72°C for 30 cycles, with a final 2min

extension at 72°C. For simplex PCR: 94°C for 2min, followed by 30 cycles of 94°C for 30sec, appropriate annealing temperature for 40sec (*irp*-1 – 61,4°C*, rmpA -50*°C, *magA* -59°C, *hlyA*- 63°C, *cnf-1*- 56°C), 72°C for 1min, and 72°C for 10min.

Hypermucoviscosity testing

Single colonies obtained after overnight culture on Mueller Hinton agar plates were tested for their ability to form viscous strings. The formation of a string greater than 5 mm in length is indicative of a hypermucoviscosity positive phenotype [7].

Clonal genetic relatedness analysis by PCR MP genotyping

We used the PCR MP method according to the procedure described by Stojowska et al.[8] with a slight modification. The denaturation temperature was calculated during the optimization experiments for several *K. pneumoniae* isolates, using a gradient range from 83.1 to 89°C; the established optimal denaturation temperature was 85.6°C. Electrophoresis of the PCR products was carried out on 6% polyacrylamide gel. Comparisons of electrophoretic profiles, based on band position, were made using MVSP 3.22 software (KCS - Kovach Computing Services) and UPGMA (unweighted pair-group method with arithmetic averages). The Pi cut-off for genotype definition was 98%.

To confirm/exclude horizontal transmission in the hospital setting, the same genotype patterns of *K. pneumoniae* were additionally tested with PCR MP at increasing denaturation temperatures (short gradient: 84.8-87.8°C).

Cluster analysis for co-occurrence of VFs

Cluster analysis was performed using MVSP software (KCS - Kovach Computing Services), version 3.22. (http://www.kovcomp.com) and an online tool for creating Venn diagrams available at http://bioinformatics.psb.ugent.be/webtools/Venn/.

Statistical analysis

All analyses were performed using Statistica 12.0 (StatSoft) software. The Mann-Whitney U test was used to compare continuous variables, and Fischer's exact test was used to compare proportions.

Logistic regression analyses were performed to find independent risk factors for AGPN. Variables with $p \leq 0.1$ in univariate analysis were introduced into multivariate stepwise logistic regression model. Associations are given as odds ratios (ORs) with a 95% confidence interval (95% CI). $P \leq 0.05$ was considered to be statistically significant.

Results

Clinical features

We analyzed episodes of *K. pneumoniae* UTI in 54 RTx recipients, including 43% of male gender, with mean age of 52.6 ± 17 years. The etiologies of ESRD were as follows: primary glomerulonephritis (13/24.1%), diabetic nephropathy (9/16.7%), autosomal dominant polycystic kidney disease (ADPKD) (8/14.8%), tubulointerstistial nephritis (6/11.1%), hypertensive nephropathy (3/5.5%), lupus nephritis (2/3.7%), others (4/7.4%), unknown etiology (9/16.7%). 45 (83.3%) patients were on maintenance hemodialysis (MHD) before the transplantation, 14 (25.9%) were on chronic peritoneal dialysis (CPD), including 5 patients who were treated consecutively with both MHD and CPD (9.3%); none of the patients underwent pre-emptive transplantation. All patients received grafts from deceased donors. There were 46 patients maintained on tacrolimus, 7 on CsA, 51 on MMF/MPS, 3 on everolimus. All patients received glucocorticosteroids. Induction with ATG was used on 3 patients, and with basiliximab in 17 patients. Double J ureteral catheters were used in 32 cases (59%). Nineteen (35%) patients had DGF and 17 (31%) had ATN. Twelve (22%) patients were diagnosed with AR and 15 (28%) developed a CMV infection.

54 RTx patients developed 61 episodes of *K. pneumoniae* UTI, with 32 cases of AB (53%), 10 cases of lower UTI (16%), 19 cases of AGPN (31%), including 6 cases of bacteraemia. Twenty two patients were diagnosed with only a single *K. pneumoniae* UTI episode. The other 32 patients (59%) experienced recurrent UTIs; and in 19 of those patients these were multiple *K. pneumoniae* UTI relapses, while 13 patients had a history of re-infections with various pathogens. 74% of UTI episodes were caused by ESBL+ strains, and we identified 2 carbepenemase producing strains. When we investigated the distribution of ESBL+ strains according to the UTI category, ESBL producing strains

showed similar distribution irrespective of UTI type with 74% in upper *K. pneumoniae* UTI, 77% of AGPN cases, and 67% cases of urosepsis. *K. pneumoniae* ESBL+ was responsible for 70% cases of lower symptomatic UTIs, and 75% of AB. Both carbapenemase producing strains were isolated in the cases of AB.

There was a significant, but transient rise in the creatinine level in upper UTIs. No kidney allograft losses due to *K. pneumoniae* infection, and no deaths were observed in the study cases.

Comparison of patient risk factors for upper *K. pneumoniae* UTIs

On multivariate analysis, the only independent risk factor for upper UTIs was urine flow impairment (hesitancy/retention symptoms in men, sex-independent urogenital surgery history and lower urinary tract malformation as underlying causes of ESRD, VUR or strictures at uretero-vesical junction), which increased the risk of AGPN over 14-fold. Almost 58% of patients with AGPN had either VUR or strictures at the uretero-vesical junction, neurogenic bladder or benign prostate hyperplasia. In patients with acquired urine flow impairment resulting from RTx the mean time from transplantation to the diagnosis of either VUR or strictures at the uretero-vesical junction was 7.7 ± 5.7 months. In most cases we searched for structural causes and confirmed the diagnosis after a second episode of AGPN. None of the following factors: female gender, comorbidity, CMV infection and history of AR emerged as independent predictors of *K. pneumoniae* upper UTIs. Tacrolimus seems to exert a protective effect, while the use of induction regimens did not alter the risk of developing *K. pneumoniae* AGPN. The same applied for the use of a double-J ureteral catheter (Table 2).

Genetic relatedness of strains

PCR MP analysis of 61 *K. pneumoniae* strains isolated from 54 RTx patients, a reference strain ATCC (700603™) and *K. pneumoniae* KPC (from another hospital used as a control) showed high diversity with 54 genotypes (Fig. 1). Out of 61 clinical isolates from RTx patients, there were 52 distinct genotypes G1-G52 (supplementary Table 1). In 5 patients (P3, P5, P16, P 23, P47) in whom we had more than one isolate, each isolate had a distinct genotype.

In 15 patients, we identified 7 closely related genotypes. Isolates Kp15 and Kp17 from patients P14 and P16 had the same G14 genotype, isolates Kp14 and Kp18 (patients P13 and 16) had the same G13 genotype, isolates Kp16 and Kp19 (patients P15 and P17) had the same G15 genotype, and isolates Kp33, Kp37, Kp40 (patients P30, P5 and P36) had the same G29 genotype, isolates Kp44 and Kp45 (patients P40 and P41) had the same G38 genotype, isolate Kp50 and Kp51 (patients P46, P47) had the same G43 genotype and patients P49 and P50 with Kp54 and Kp55 isolates also had the same genotype G46.

PCR MP analysis at increasing denaturation temperatures confirmed these findings, and the presence of hospital-acquired infections. There were two strains with distinct genotypes and distinct VFs profiles isolated at the same time from patient P16. However, these strains had genotypes concordant with the genotypes of 3 other strains isolated from 3 different patients a month earlier (genotype G14 isolated from patient P14; genotype G13 from patients P12, P13). All of these isolates with closely related genotypes were ESBL-producing.

Comparison of virulence factors for upper *K. pneumoniae* UTIs

Type 1 and type 3 fimbriae are responsible for the adherence to the uroepithelial cells and the biofilm formation [9]. *mrk*D and *fim*H-1 genes encoding type 1 and 3 fimbriae are typically found in uropathogenic *K. pneumoniae* strains [10,11] and were also present in most of our isolates. *mrkD* was the most prevalent VF gene in strains isolated from RTx patients and was found in 86.9% isolates. Yersiniabactin is essential for biofilm formation in the bladder and has a protective function against intracellular copper toxicity [12]. Yersiniabactin gene (*irp*2) was detected with approximately a 3-fold higher prevalence than previously reported, i.e. in 61.1% of our strains [13,14]. So far yersiniabactin production was mainly observed in blood and lung isolates, seldom in urine and wound [11,15]. In our study, in 3 cases of urosepsis, blood and urine isolates had the some genotype and 2 out of 3 carried yersiniabactin genes.

On univariate analysis *kpn* gene predominated in cases of AB when compared to symptomatic UTIs and *fyuA* was more common in upper UTIs than in cases of AB (Supplementary Table 2). We found no

association between VFs profile and the type of UTI (lower UTI vs. upper UTI) in the multivariate analysis in RTx patients. Of note, *magA, hlyA,* which were not found in any isolates, *cnf-1* and *rmpA* present only in 3 and 4 isolates, respectively, and *mrkD* identified in 53 out of 61 strains were not included in the above-mentioned analysis. We also found no significant difference when we compared the total number of identified VFs genes between isolates from cases of lower and upper UTIs in RTx patients. The median number of VFs was 7 in both groups (p = 0.42) with lower quartile of 3 and upper quartile of 8 for RTx patients and 6 and 9 in non-RTx group, respectively. Also after further stratification of data into cases of AB, lower UTI and upper UTI we found no significant difference in the total number of VFs genes identified between the different subgroups (Supplementary Table 3-4).

Immunosuppression and virulence factors

Further analysis showed that the prevalence of the *uge* gene was lower in RTx patients on everolimus when compared to isolates from patients not receiving mTOR inhibitors (33.3% vs 82.8% p<0.05). *ycfM* – outer membrane lipoprotein, was less common in RTx patients who received basiliximab when compared to non-induction protocols (52.6% vs 76.2% p=0.08). In patients who received ATG as induction *iutA* – aerobactin was present in 75% of strains and *entB* – enterobactin in 25% of isolates vs 24.6% (p=0.06) and 77.2% (p=0.05), respectively, when no lymphocyte depleting induction was used. All these differences were irrespective of the UTI type and neither reached statistical significance.

Comparison of virulence factors between RTx and non-RTx populations

We compared the prevalence of virulence genes in the 61 bacterial isolates from 54 RTx patients, and 36 non-RTx patients (one episode for each patient) (Table 3).

The hypermucoviscosity phenotype (HMV) is associated with *mag*A and *rmp*A genes, and predominates in patients with invasive diseases, especially liver abscess [16,17]. We found the HMV phenotype and the associated *rmp*A gene (an enhancer of the colony mucoidy) only in two strains

(6.6 % of isolates) from RTx patients, and one from non-RTx patients, and none of our isolates had the *mag*A gene encoding a capsular polysaccharide polymer.

Cytotoxic necrotizing factor-1 (*cnf*-1 gene) and haemolysin toxin protein (*hlyA* gene) are highly pathogenic VFs common in uropathogenic *E.coli* strains (UPEC) [18,19]. No strain showed hemolytic activity, and *hly*A was detected only in 2 cases in non-RTx patients. Similarly, the *cnf*-1 gene was seldom found in our material.

The other genes, *uge* and *ycf*M encoding capsule lipoprotein and external membrane protein, promote infection by resistance to phagocytosis and mediate binding to the extracellular matrix, and were found in *K. pneumoniae* urine isolates from the non-RTx population [20]. We confirmed their presence in urine isolates from both RTx patients, and the control group. The *uge* gene was detected in as many as 80.3 % of isolates. In both groups we identified all genes encoding VFs responsible for adhesion and biofilm formation in approximately half of strains.

Siderophores (yersiniabactin, aerobactin, enterobactin) are iron uptake systems that help extracellular pathogens like *Klebsiella s*pp. to overcome host iron restriction. Siderophores increase bacterial pathogenicity by causing iron deficiency in the host and therefore leading to a significant reduction in humoral defense mechanisms. The unidirectional analysis of PCR-based detection of genes encoding the examined *K. pneumoniae* virulence factors (Table 3) showed that *ent* B - the enterobactin biosynthesis gene (a catecholate siderophore) predominated in strains isolated from RTx patients when compared to isolates from non-RTx patients (p= 0.015). On the contrary, *iut*A aerobactin gene (a hydroxamate siderophore) predominated in isolates from non-RTx patients (p = 0.005).

After further stratification of data into cases of AB, lower UTI and upper UTI, *ent* B - the enterobactin biosynthesis gene predominated in strains isolated from RTx patients when compared to isolates from non-RTx patients only in cases of AB. *iroN* salmochelin gene was significantly more common in lower UTIs in RTx patients, while *iut*A aerobactin gene predominated in isolates from non-RTx patients with upper UTIs (Supplementary Tables 5-7).

Because one-dimensional analysis of *K. pneumoniae* VFs in RTx and non-RTx populations was able to identify significant differences only in the prevalence of *entB* gene and *iutA* gene, we performed a clustering analysis for co-occurrence of VFs. The VFs combinations are presented in Figure 2a for RTx patients and Figure 2b for non-RTx patients. *mrk*D, *kpn* and *fim*H-1 genes encoding type 1 and 3 fimbriae was the most commonly identified VFs cluster for strains isolated from both RTx and non-Rtx patients and was found in 85-90% of cases. This points to the role of fimbriae in urinary tract colonization irrespective of RTx status.

Only 45% of isolates from RTx recipients showed clustering of various siderophore types i.e. aerobactin, yersiniabactin and enterobactin. There was relatively strong evidence of clustering between the enterobactin encoding gene *entB* and *ycfM* gene. Outer membrane lipoprotein encoded by *ycfM* gene assists adhesion and biofilm formation. Enterobactin (encoded by *entB* gene) apart from iron acquisition (mainly from transferrin), also facilitates biofilm formation and maturation. The co-occurrence of *entB* and *ycfM* may indicate the ability of a strain to form biofilm within the urinary tract. The combination of *entB* with various genes encoding adhesins: *uge, mrkD, kpn* and *fimH-1* was found in 83% of strains from RTx patients.

In the RTx population, less than 10% of strains had a cluster of five genes associated with four bacterial iron acquisition systems i.e. enterobactin (*entB* - enterobactin gene), salmochelin (*iroN* gene - siderophore receptor), yersiniabactin (*irp-1* gene and *fyuA* gene), aerobactin (*iutA* gene – siderophore receptor), while such a cluster was present in over 20% of isolates from non-RTx patients (Table 4, Figure 3). This difference did not reach statistical significance. However, a co-occurrence of two sets of four genes encoding siderophores: *entB, fyuA, irp-1, iroN,* and *entB, fyuA, irp-1, iutA* differed significantly between groups and was more prevalent in the non-RTx population. In RTx patients a cluster of consisting of *fyuA, irp-1, iroN, iutA* was present in 23% of strains, while it was uncommon in the non-RTx group ($p<0.05$).

hlyA (α-hemolysin) and *rmpA* (regulator of mucoid phenotype A) genes were in general infrequent. However, in the control group (non-RTx) the co-occurrence rate for these two genes was 40% indicating highly pathogenic strains.

Discussion

To determine the risk factors for the progression from *K. pneumoniae* lower UTIs to AGPN, we have prospectively studied the characteristics of *K. pneumoniae* strains causing UTIs and their relationship with UTI severity. 31% of all diagnosed UTIs were cases of AGPN and almost 10% were accompanied by bacteremia. Over 70% of strains were ESBL positive. Linares et al. analyzed *K. pneumoniae* infections in more than 1000 solid organ transplant recipients to identify the urinary tract as the major site of infection in 72% of cases [21]. Only slightly over 50% of isolates were ESBL-positive, in contrast to our findings. Holt et al. recently reported that nosocomial carriage isolates had more acquired antimicrobial resistance genes than community carriage isolates; possibly due to selection from antimicrobial exposure during hospital treatment [22]. In aggregate the studies by Linares and colleagues and other authors pointed out that median time from transplantation was shorter in infections due to resistant ESBL-producing *K. pneumoniae* in comparison to ESBL-negative pathogens, which in general supports the horizontal transmission of ESBL-producing *K. pneumoniae* during hospital stay [23,24]. However, it is a known fact that the epidemiology of multidrug resistant bacteria is changing. Not only has the total number of ESBL-positive strains substantially increased, but these pathogens are no longer confined to the hospital setting [25].

K. pneumoniae can cause both community-acquired infections and hospital-acquired infections. Hospital-acquired infection-associated clones usually lack virulence genes but are multidrug resistant. Multilocus sequence typing (MLST) is a nucleotide sequence method based on polymorphisms of seven housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB, and tonB*) that is adequate for characterizing the genetic relationships among bacterial isolates and was shown to confirm strain concordance with the power of whole genome sequencing [26]. MLST has been used to determine clonal background in order to identify and track KPC-producing, especially ST258, and

colistin-resistant strains [27,28]. There were outbreaks of *K. pneumoniae* ST11 New Delhi metallo-β-lactamase (NDM)-1 in Poland, when sequence type was confirmed with MLST. There was also a sporadic case of KPC-2-producing *K. pneumoniae* identified, which belonged to ST45 and substantially differed from the *K. pneumoniae* ST11 outbreak isolates [29]. *K. pneumoniae* ST11 NDM-1 strains were also described in Greece [30], Czech Republic and Slovakia [31]. However, the subclones had different genetic profiles with different types of β-lactamases or carbapenemases.

We analyzed strains isolated from patients hospitalized in a single department of one hospital. Most of the isolates were resistant to beta-lactams, and only two were carbapenemase-producing. Our main aim was to find out if strains isolated from RTx patients were invasive community-acquired high virulence strains or hospital-acquired low virulence isolates. Holt et al. suggested that virulence was not dependent on strain genetic background and is not defined by the sequence type [22]. For that reason, we did not use MLST method to identify particular sequence types or clonal complexes, but decided to use PCR melting profiles (PCR-MP) method. PCR-MP has a high discriminatory power and can be useful for epidemiological studies of genetic relationship of strains isolated from a single unit over a short or long period of time in order to find the source, reservoirs and how infection spread [8].

We had very few cases of horizontal transmission in the hospital setting, confirmed by clonal genetic relatedness evaluation. The typing results showed a high diversity. Forty five unique genotypes of clinical *K. pneumoniae* strains may suggest urinary tract colonization by commensal strains, e.g. from the gastrointestinal tract, or ascending non-nosocomial infection. However, this requires verification by comparison of genotypes with those of stool or anal swab isolates. ESBL-producing isolates from 15 patients had 7 closely related genotypes indicating nosocomial infections. Another two patients underwent three independent *K. pneumoniae* UTI episodes. Each isolate had a different genotype, and different VFs profiles, suggesting re-infections as opposed to relapse. Genotype distribution did

not have significant associations with heterogeneous VFs profiles. Recently, Holt et al. showed a huge variation of *K. pneumoniae* genotypes, with the possibility to gain or lose a whole gene [22].

We investigated the presence of VFs genes that represent each of the four major virulence factors classes that have been well characterized in *K. pneumoniae:* the fimbriae, siderophores, capsule and lipopolysaccharide. *fim*H and *uge* genes, were detected in all *K. pneumoniae* isolates collected from inpatients submitted to a renal transplant unit in a study by Calhau et al [32]. The presence of the other above-mentioned virulence encoding-genes has not been analyzed in the RTx population so far, we are the first to report it.

Siderophores, together with the host's response can potentially modulate iron availability. When we took an in-depth look into bacterial iron acquisition systems, we found significant differences between isolates from RTx patients and the control group, perhaps reflecting disordered iron balance in RTx recipients [33,34]. *ent* B - the enterobactin biosynthesis gene was significantly more prevalent in strains isolated from RTx patients, while the *iut*A aerobactin gene predominated in isolates from non-RTx patients. Aerobactin was the only siderophore showed to account for the enhanced virulence of a hypervirulent *K. pneumoniae* (hvKP) [35,36]. On the other hand, enterobactin has a higher affinity for iron compared to other chelators [37], and is also the only siderophore, shown to inhibit myeloperoxidase (MPO), a bactericidal enzyme released by the host neutrophils [38]. Along with MPO neutrophils secrete the siderophore-binding protein lipocalin 2, which does not bind any hydroxamate-type ferric siderophores such as yersiniabactin or mixed type siderophores such as aerobactin, but chelates catecholate-type ferric siderophores such as enterobactin and salmochelin [39]. Yet, enterobactin and salmochelin are not functionally equivalent. Structural and functional variety of siderophores results in altered susceptibility to the host’s defense mechanisms, e.g. lipocalin 2 shows a much higher affinity for enterobactin than for its glycosylated form salmochelin [40].

The clustering between various types of siderophores was also much more common in strains isolated from non-RTx patients. In aggregate, these results indicate that lower virulence may be required for a strain to be pathogenic in RTx recipients.

Another interesting finding was that the prevalence of various virulence genes seemed to be affected by the choice of immunosuppressive regimen. This suggests that the type or strength of immunosuppression used may influence the selection of strains with a particular virulence factor profile.

We failed to find an association between the presence of any investigated genes encoding VFs and the development of upper *K. pneumoniae* UTIs. This is consistent with the observation that *K. pneumoniae* hospital-acquired infections with multi-drug resistant strains, are likely to depend more on host factors, such as compromised immunity, than on specific pathogenicity factors in the bacterium; as opposed to community-acquired invasive infections [22].

We identified only one predictive variable for the development of AGPN, and this was urine flow impairment of different mentioned-above causes (Table 2). The likelihood of AGPN development was 14-fold higher in patients with VUR, or strictures at the uretero-vesical junction, neurogenic bladder or benign prostate hyperplasia. Active reflux has long been reported as being significantly associated with poor graft outcome [41,42]. On the other hand, Margreiter et al. reported that VUR was confirmed by voiding cystourethrography in 40% of 646 consecutive RTx recipients, and did not affect the incidence of UTIs [43]. In a retrospective cohort study of 23,622 adult male primary RTx recipients, benign prostate hyperplasia was independently associated with recurrent UTIs [44]. One of the findings of the study by Bodro et al., that is in accordance with our observations, was that while classic risk factors for UTI (female gender and diabetes) were absent, the variables associated with a higher risk of recurrent UTI were: a first or second episode of infection by multi-drug resistant bacteria, age >60 years, and re-operation [45]. However, the authors concluded that re-operation reflected a more intense hospital exposure, not an underlying problem with urine flow. Considering the significant influence of urinary flow abnormalities on the likelihood of AGPN development, we

would strongly recommend examination for VUR, or urine flow obstruction at the first AGPN, especially with *K. pneumoniae* as the causative agent.

Another interesting finding is what seems to be a protective effect of tacrolimus based immunosuppression. The underlying mechanism is not clear. It has been previously shown that cyclosporine seemed to promote a higher expression of enterococcal virulence factor PBP5 as compared to tacrolimus [46]. However, this effect of calcineurin inhibitors has not been studied so far in *Enterobacteriaceae*.

Of note, there was a very high recurrence rate in the case of *K. pneumoniae* UTIs. 60% of our patients had a history of recurrent UTIs. *K. pneumoniae* has been shown earlier to be the most common pathogen in recurrent UTIs during the first year after RTx [4]. In a recently published analysis of the impact of antibiotic resistance on the development of recurrent and relapsing symptomatic UTIs in RTx, ESBL-producing *K. pneumoniae* was the pathogen most commonly isolated in recurrent UTIs in this population [47].

Several limitations of this study deserve mention. The study was restricted to a single center with particular strategies for prophylaxis and treatment, and had limited power to identify statistically significant differences because of the small number of cases included. The discrepancy in the number of cases between RTx and non-RTx patients could have resulted from the fact that only hospitalized patients were included in the analysis. The decision on hospital admission is made at the discretion of the attending physician. RTx patients are more likely to be hospitalized because of the burden of immunosuppression and usually higher number of comorbidities than in case of non-RTx patients. We analyzed only a limited number of genes encoding VFs, some of which turned out to be very common and detectable in a substantial number of strains, or were not detected at all. What is more, we only identified VFs genes presence, but did not confirm their expression. Despite these limitations, we believe that our study is of importance, as data regarding *K. pneumoniae* UTIs in the RTx population is scarce.

Summarizing, we would like to highlight the following: *K. pneumoniae* upper UTI is very suggestive of urine flow impairment, e.g. VUR or strictures at the uretero-vesical junction, urinary tract malformation, neurogenic bladder or benign prostate hyperplasia. This confirms that opportunistic *K. pneumoniae* infections likely depend more on host factors, than on specific pathogenicity factors in the bacterium. However, the type or strength of immunosuppression used may influence the selection of strains with a particular virulence factor profile. There were no significant associations between the genotypes of *K. pneumoniae* UTI and their VFs profiles. This confirms the horizontal transfer of VFs genes on mobile genetic elements called pathogenicity islands [48].

Authorship:

Justyna E. Gołębiewska - Concept/design, Data collection, Data analysis/interpretation, Drafting article, Statistics;

Beata Krawczyk - Concept/design, Laboratory analyses, Data analysis/interpretation, Drafting article;

Magdalena Wysocka, - Laboratory analyses, Data analysis/interpretation, Drafting article;

Aleksandra Ewiak, Jolanta Komarnicka, Marek Bronk – Strains collection, Laboratory analyses;

Bolesław Rutkowski, Alicja Dębska-Ślizień - Critical revision of article, Approval of article;

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Ethical statement:

This study was approved by the ethical committee of the Medical University of Gdańsk (NKBBN/510/2015).

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Table 1. Characteristics of the selected virulence factors and PCR products

Virulence genes and their functions		**PCR products [bp]**	**Primers reference**
	Multiplex I		
fimH-1	Gene encoding mannose-specific adhesin sub-unit of Type 1 fimbriae. Type 1 fimbriae, and the adhesive subunit FimH in particular, play an important role in UTI caused by *K. pneumoniae* and is involved in biofilm formation	688	15
uge	Gene encoding uridine diphosphate galacturonate 4-epimerase essential for capsule and smooth lipopolysaccharide synthesis.	534	49
irp-2	Siderophore -promotes biofilm formation under iron-depleted conditions	287	13
	Multiplex II		
kpn	FimH-like adhesin	626	15
mrkD	Type 3 fimbriae is responsible for the adherence to epithelial cells of the urogenital tract and play an important role in the first step of biofilm formation	240	50
ycfM	Outer membrane lipoprotein	160	15
	Simplex PCR		
rmpA	Regulator of mucoid phenotype A	535	20

magA	Mucoviscosity-associated gene A	1282	51
hlyA	α-hemolysin, a cytolytic pore-forming toxin which makes available nutrients such as the haemoglobin iron	1177	52
cnf-1	Cytotoxic necrotizing factor-1, causes constitutive activation of GTPases altering host cell actin cytoskeleton, and promotes bacterial invasion	498	52
irp-1	Yersiniabactin (a phenolate siderophore) biosynthesis gene	238	13
fyuA	Yersiniabactin (a phenolate siderophore) receptor gene	547	13
entB	Enterobactin (a catecholate siderophore) biosynthesis gene; promotes biofilm development and maturation	371	15
iutA	Aerobactin (a mixed type siderophore) receptor gene	300	52
iroN	Salmochelin (a catecholate siderophore) receptor gene	665	52

Table 2. Univariate analysis of risk factors associated with the development of acute graft pyelonephritis.

Variable, n (%)	Univariate analysis OR (95% CI)	p	Multivariate analysis OR (95% CI)	p
Age (years)	1.02 (0.99 – 1.06)	0.22		
Gender (F/M)	0.4 (0.13 – 1.22)	0.11		
Comorbidity (CCI) (points)	1.21 (0.93 – 1.57)	0.15		
Recurrent UTIs before RTx	1.13 (0.30 – 4.35)	0.86		
HD before RTx	0.89 (0.20 – 4.00)	0.88		
PD before RTx	1.69 (0.50 – 5.71)	0.40		
AR	2.77 (0.76 – 10.13)	0.13		
ATN	1.46 (0.46 – 4.60)	0.52		
DGF	1.62 (0.53 – 4.98)	0.40		
Double-J catheter	1.14 (0.38 – 3.40)	0.82		
MMF	0.43 (0.06 – 3.27)	0.42		
CsA	2.53 (0.56 – 11.46)	0.23		
Tac	0.30 (0.07 – 1.26)	0.10	0.08 (0.01-0.53)	<0.01
Everolimus	1.11 (0.10 – 13.06)	0.93		
induction (either ATG or basiliximab)	0.75 (0.24 – 2.37)	0.62		
CMV infection	1.64 (0.52 – 5.24)	0.40		
urine flow impairment	6.88 (2.03 – 23.28)	0.001	14.28 (2.7 -75.56)	0.002
fimH-1	1.26 (0.29 – 5.37)	0.76		
kpn	0.27 (0.08 – 0.93)	0.04	0.26 (0.05 – 1.32)	0.1

mrkD	N/A			
ycfM	0.35 (0.11 – 1.09)	0.07	0.41 (0.08 – 2.08)	0.28
uge	1.46 (0.35 – 6.12)	0.60		
rmpA	N/A			
magA	N/A			
hlyA	N/A			
cnf-1	N/A			
irp-1	1.66 (0.56 – 4.98)	0.36		
irp-2	1.35 (0.45 – 3.99)	0.59		
fyuA	2.79 (0.91 – 8.55)	0.07	2.07 (0.5 – 8.57)	0.32
entB	0.68 (0.2 – 2.25)	0.53		
iutA	0.38 (0.09 – 1.51)	0.14		
iroN	0.60 (0.15 – 2.49)	0.47		

RTx – renal transplantation, CCI – Charlson Comorbidity Index, HD – haemodialysis, PD – peritoneal dialysis, AR – acute rejection, ATN – acute tubular necrosis, DGF – delayed graft function, CsA – cyclosporine, MMF – mycophenolate mofetil, tac – tacrolimus, CMV – cytomegalovirus

Table 3. Comparison of bacterial virulence genes in *K. pneumoniae* isolates derived from RTx and non-RTx patients

Variable, n (%)	**RTx (n=61)**	**Non-RTx (n=36)**	**p**
fimH-1	50 (82%)	32 (88.9%)	0.56
kpn	46 (75.4%)	31 (86.1%)	0.3
*mrk*D	53 (86.9%)	32 (88.9%)	1.0
*ycf*M	42 (68.9%)	24 (66.7%)	0.83
uge	49 (80.3%)	27 (75%)	0.61
*rmp*A	4 (6.6%)	1 (2.8%)	0.65
*mag*A	0 (0%)	0 (0%)	N/A
*hly*A	0 (0%)	2 (5.6%)	0.14
cnf-1	3 (4.9%)	5 (13.9%)	0.14
irp-2	29 (61.1%)	22 (47.5%)	0.21
irp-1	30 (49.2%)	20 (55.6%)	0.67
fyuA	28 (45.9%)	18 (50%)	0.83
entB	**45 (73.8%)**	**17 (47.2%)**	**0.015**
iutA	**17 (27.9%)**	**21 (58.3%)**	**0.005**
iroN	13 (21.3%)	8 (22.2%)	1.0

Table 4. Co-occurrence of siderophore system encoding genes among *K. pneumoniae* isolates from RTx and non-RTx patients.

Isolates from RTx patients [n=61]			**Isolates from non-Rtx patients [n=36]**			
co-occurrence of siderophores	Number of strains [n]	% of strains	co-occurrence of siderophores	Number of strains [n]	% of strains	**P value**
entB fyuA iroN irp-1 iutA	6	9.84	entB fyuA iroN irp-1 iutA	8	22.22	0.13
entB fyuA iroN irp-1	9	14.75	entB fyuA iroN irp-1	3	8.33	0.53
entB fyuA iroN iutA	1	1.64	entB fyuA iroN iutA	3	8.33	0.14
entB fyuA iroN irp-1	**0**	**0**	**entB fyuA iroN irp-1**	**3**	**8.33**	**0.048**
entB fyuA irp-1 iutA	**2**	**3.28**	**entB fyuA irp-1 iutA**	**6**	**16.7**	**0.048**
entB iroN irp-1 iutA	1	1.64	entB iroN irp-1 iutA	0	0	1.0
fyuA iroN irp-1 iutA	**14**	**22.95**	**fyuA iroN irp-1 iutA**	**2**	**5.55**	**0.045**
entB fyuA irp-1	13	21.31	entB fyuA irp-1	3	8.33	0.16
entB iroN iutA	5	8.20	entB iroN iutA	0	0	0.15
entB irp-1 iutA	1	1.64	entB irp-1 iutA	1	2.77	1.0
entB fyuA iroN	0	0	entB fyuA iroN	2	5.55	0.14
entB fyuA iutA	0	0	entB fyuA iutA	1	2.77	0.37
fyuA ironN irp-1	1	1.64	fyuA ironN irp-1	1	2.77	1.0
fyuA iroN iutA	2	3.28	fyuA iroN iutA	2	5.55	0.63
fyuA irp-1 iutA	5	8.20	fyuA irp-1 iutA	3	8.33	1.0
iroN irp-1 iutA	1	1.64	iroN irp-1 iutA	1	2.77	1.0
entB fyuA	1	1.64	entB fyuA	1	2.77	1.0
entB iroN	5	8.2	entB iroN	3	8.33	1.0
entB irp-1	2	3.28	entB irp-1	2	5.55	0.63
entB iutA	1	1.64	entB iutA	1	2.77	1.0
fyuA iroN	1	1.64	fyuA iroN	1	2.77	1.0

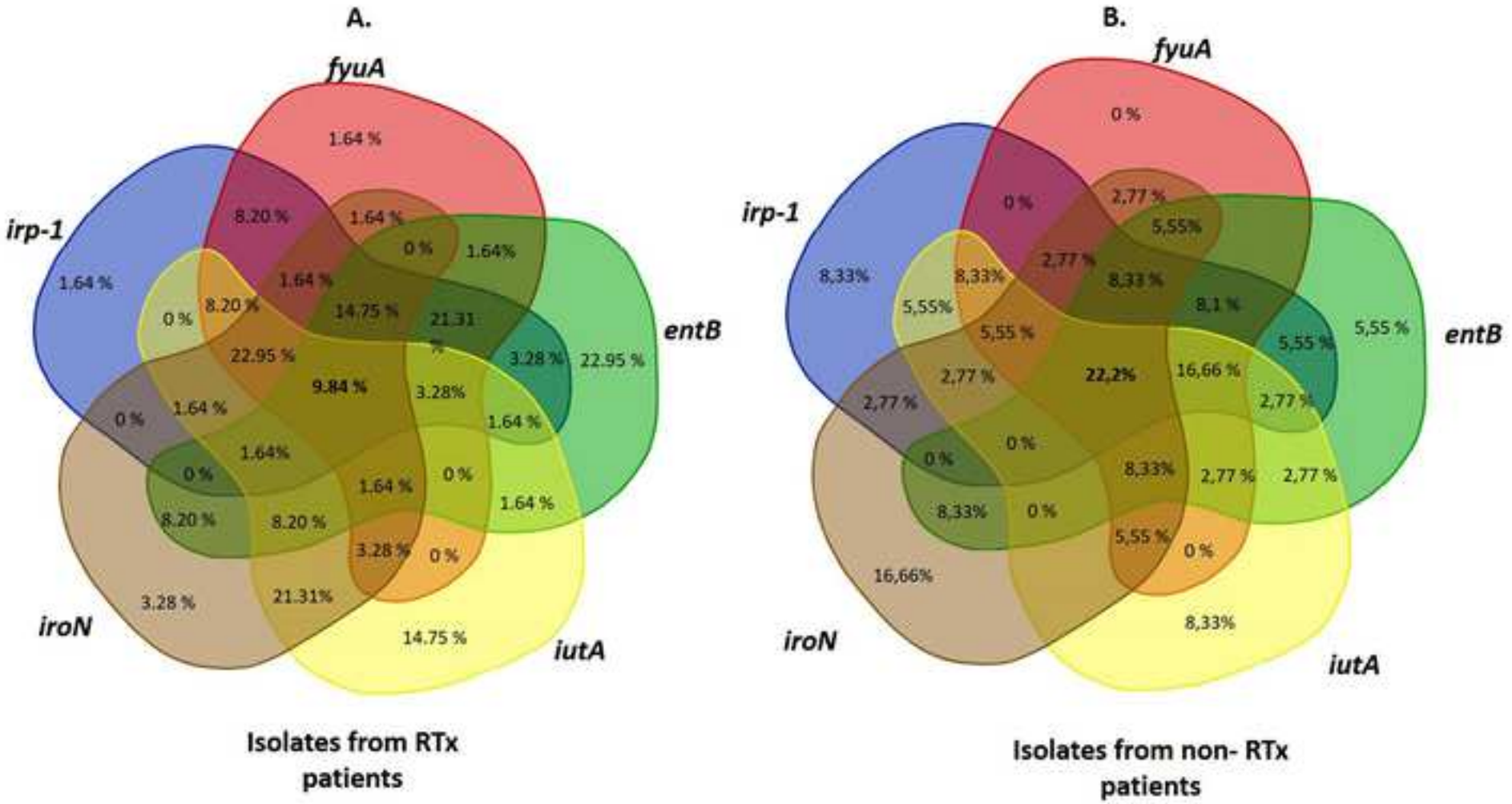
fyuA irp-1	5	8.20	fyuA irp-1	0	0	0.15
iroN iutA	13	21.31	iroN iutA	3	8.33	0.16
iroN irp-1	0	0	iroN irp-1	1	2.77	0.37
irp-1 iutA	0	0	irp-1 iutA	2	5.55	0.14
entB	**14**	**22.95**	**entB**	**2**	**5.55**	**0.045**
fyuA	1	1.64	fyuA	0	0	1.0
iroN	**2**	**3.28**	**iroN**	**6**	**16.21**	**0.048**
irp-1	1	1.64	irp-1	3	8.33	0.14
iutA	9	14.75	iutA	3	8.33	0.53

Figure 1. Genetic relatedness of of 61 *K. pneumoniae* strains isolated from 54 RTx patients, a reference strain ATCC (700603™) and *K. pneumoniae* KPC (from another hospital used as a control)

Figure 2. Co-occurrence of virulence factors encoding genes among *K. pneumoniae* isolates from (A) RTx and (B) non-RTx patients.

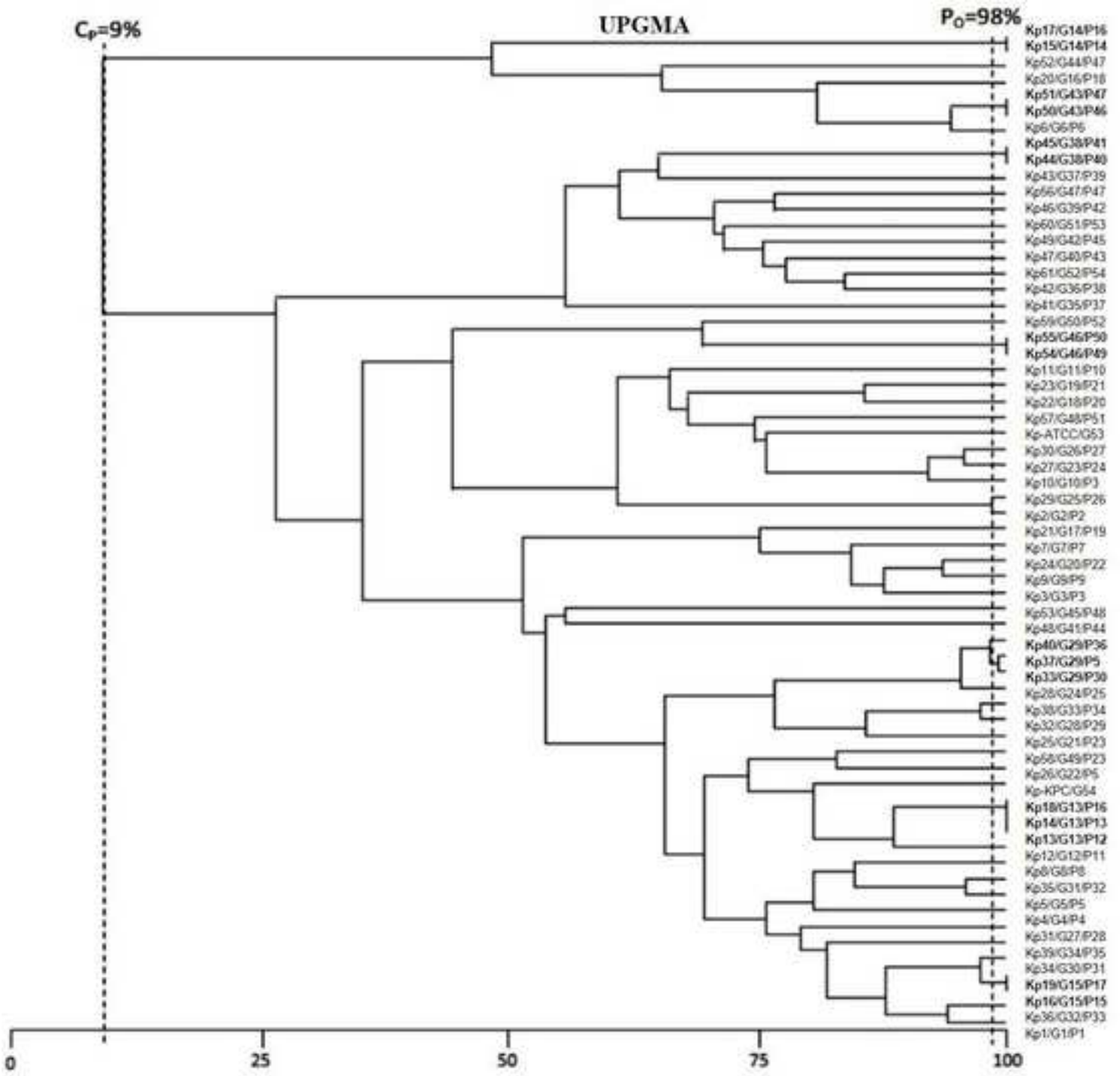
Figure 3. Co-occurrence of siderophore system encoding genes among *K. pneumoniae* isolates from (A) RTx and (B) non-RTx patients.

Figure



Figure

Click here to access/download;Figure;Fig. 1.jpg



Figure

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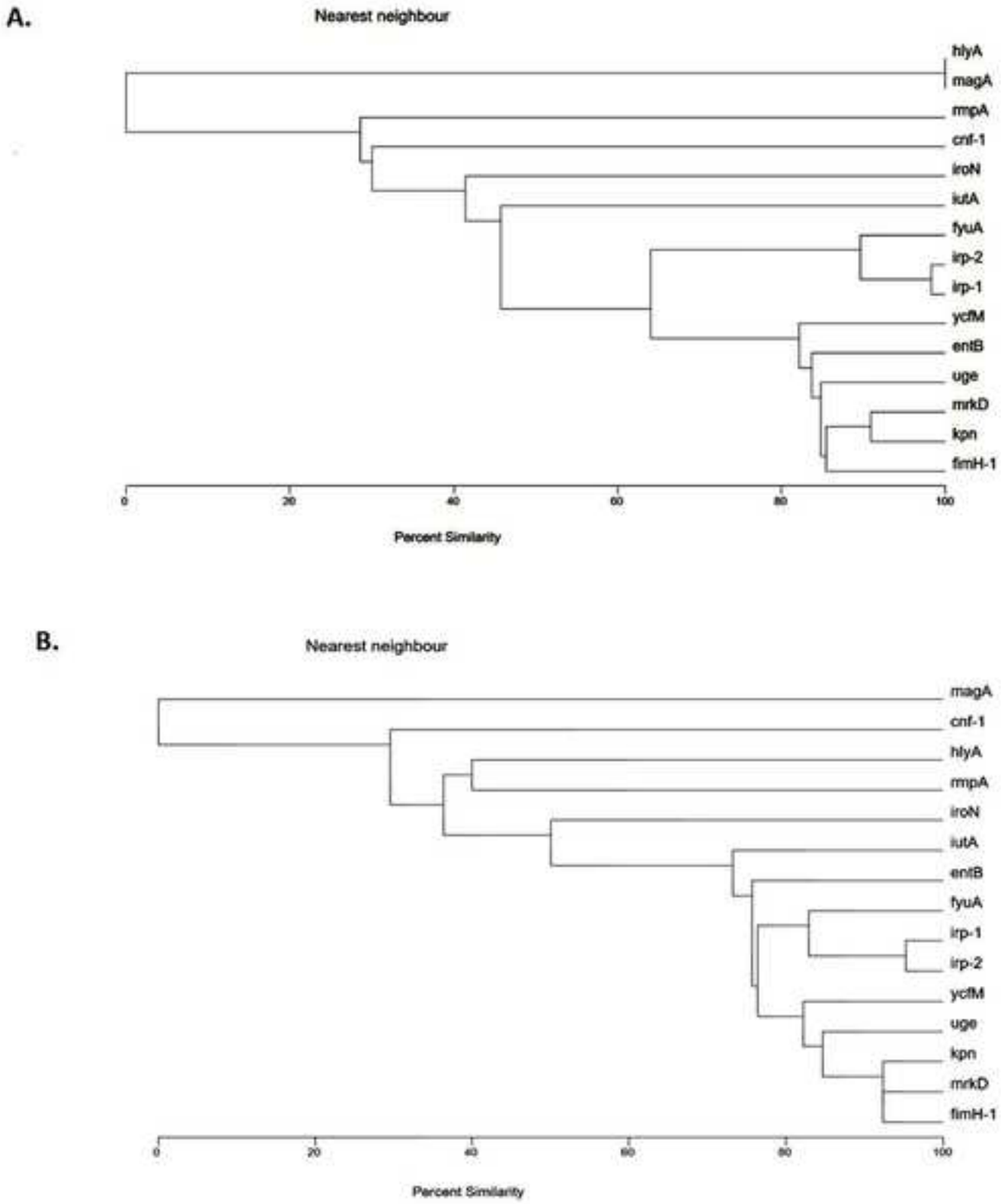

Supplementary Table 1. Genotypes and virulence factors of *K.pneumoniae* strains isolated from RTx patients

N^0 of isolate (Kp)	N^0 of patient (P)	Date of isolation d/m/y	genotype	*Virulence factors*														
				fimH-1	*uge*	*kpn*	*mrkD*	*ycfM*	*rmpA*	*magA*	*hlyA*	*cnf-1*	*irp-1*	*irp-2*	*fyuA*	*entB*	*iutA*	*iroN*
Kp1	P1	24/03/2013	G1	1	1	1	1	1	0	0	0	0	1	1	1	1	0	1
Kp	P2	17/04/2013	G2	1	1	1	1	1	0	0	0	0	0	0	0	1	0	1
Kp3	P3	17/04/2013	G3	0	1	0	0	1	0	0	0	0	1	1	1	1	0	0
Kp4	P4	01/05/2013	G4	1	1	1	1	1	0	0	0	0	1	1	1	1	0	1
Kp5	P5	03/05/2013	G5	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0
Kp6	P6	09/06/2013	G6	1	1	0	1	1	0	0	0	0	1	0	1	1	0	0
Kp7	P7	12/06/2013	G7	1	1	1	1	1	0	0	0	0	1	1	1	1	0	1
Kp8	P8	25/06/2013	G8	0	0	1	1	1	0	0	0	0	1	1	1	1	0	1
Kp9	P9	04/07/2013	G9	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Kp10	P3	28/07/2013	G10	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0
Kp11	P10	08/08/2013	G11	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0
Kp12	P11	29/08/2013	G12	0	1	0	1	0	0	0	0	0	1	1	1	1	1	1

Kp13	P12	02/09/2013	G13	0	1	0	1	0	0	0	0	0	1	1	1	1	0	0
Kp14	P13	06/09/2013	G13	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1
Kp15	P14	26/09/2013	G14	1	0	1	1	1	0	0	0	0	0	0	0	0	1	0
Kp16	P15	02/10/2013	G15	1	1	1	1	1	0	0	0	0	1	1	1	1	0	1
Kp17	P16	21/10/2013	G14	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1
Kp18		21/10/2013	G13	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0
Kp19	P17	28/10/2013	G15	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Kp20	P18	05/11/2013	G16	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Kp21	P19	05/11/2013	G17	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Kp22	P20	19/11/2013	G18	1	1	0	1	1	0	0	0	0	0	0	0	1	0	0
Kp23	P21	13/01/2014	G19	1	1	0	1	1	0	0	0	0	0	0	0	1	1	1
Kp24	P22	21/01/2014	G20	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1
Kp25	P23	09/02/2014	G21	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0
Kp26	P5	10/02/2014	G22	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0
Kp27	P24	12/02/2014	G23	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0
Kp28	P25	19/02/2014	G24	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0
Kp29	P26	22/02/2014	G25	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0

Kp30	P27	12/03/2014	G26	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0
Kp31	P28	09/05/2014	G27	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Kp32	P29	07/07/2014	G28	1	1	1	0	1	0	0	0	0	1	1	0	1	0	0
Kp33	P30	09/08/2014	G29	1	0	1	1	1	0	0	0	0	0	0	0	1	0	0
Kp34	P31	30/08/2014	G30	1	1	1	1	0	0	0	0	0	1	1	1	1	0	0
Kp35	P32	07/09/2014	G31	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0
Kp36	P33	30/09/2014	G32	1	1	0	1	0	0	0	0	0	1	1	1	1	0	1
Kp37	P5	30/09/2014	G29	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Kp38	P34	08/12/2014	G33	0	0	1	1	1	0	0	0	0	1	1	0	1	0	0
Kp39	P35	11/12/2014	G34	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
Kp40	P36	14/12/2014	G29	0	1	1	1	1	0	0	0	0	0	0	0	1	1	1
KP41	P37	15/12/2014	G35	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Kp42	P38	27/11/2017	G36	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Kp43	P39	06/12/2017	G37	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Kp44	P40	12/12/2017	G38	1	0	0	0	0	0	0	0	0	1	1	1	0	1	0
Kp45	P41	03/01/2018	G38	1	0	1	1	0	0	0	0	0	1	1	1	1	1	0
Kp46	P42	14/01/2018	G39	1	1	1	1	1	1	0	0	1	1	1	1	0	1	0

Kp47	P43	15/01/2018	G40	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0
Kp48	P44	23/01/2018	G41	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0
Kp49	P45	05/02/2018	G42	1	1	1	1	1	0	0	0	0	1	1	0	1	1	0
Kp50	P46	07/03/2018	G43	1	1	1	1	0	0	0	0	0	1	1	1	0	1	0
Kp51	P47	07/03/2018	G43	1	0	1	1	1	1	0	0	0	0	0	1	1	0	0
Kp52		07/03/2018	G44	0	0	1	1	1	0	0	0	1	0	0	0	0	1	0
Kp53	P48	17/03/2018	G45	1	1	1	1	1	0	0	0	1	1	1	1	1	1	0
Kp54	P49	27/03/2018	G46	1	1	1	1	0	0	0	0	0	0	0	0	1	1	0
Kp55	P50	18/04/2018	G46	1	1	1	1	0	0	0	0	0	1	1	1	0	1	0
Kp56	P47	20/04/2018	G47	1	1	1	1	0	0	0	0	0	1	1	1	0	0	0
Kp57	P51	08/07/2018	G48	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Kp58	P23	09/07/2018	G49	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Kp59	P52	18/07/2018	G50	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Kp60	P53	19/07/2018	G51	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Kp61	P54	19/07/2018	G52	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0

Supplementary Table 2. Comparison of bacterial virulence genes in *K. pneumoniae* isolates derived from RTx patients according to type of UTI ([1]asymptomatic bacteriuria vs lower UTI, [2]asymptomatic bacteriuria vs upper UTI, [3]lower UTI vs upper UTI)

Variable, n (%)	**AB (n=32)**	**Lower UTI (n=10)**	**Upper UTI (n=19)**	**p**
fimH-1	27 (84.4%)	7 (70%)	16 (84.2%)	[1]0.37
				[2]1.0
				[3]0.63
kpn	29 (90.6%)	6 (60.0%)	11 (57.9%)	[1]1.0
				[2]0.01
				[3]1.0
*mrk*D	30 (93.8%)	8 (80.0%)	15 (79.0%)	[1]0.24
				[2]0.18
				[3]1.0
*ycf*M	24 (75%)	8 (80.0%)	10 (52.6%)	[1]1.0
				[2]0.13
				[3]0.23
uge	25 (78.1%)	8 (80.0%)	16 (84.2%)	[1]1.0
				[2]0.75
				[3]1.0
*rmp*A	3 (9.4%)	0 (0%)	1 (5.3%)	[1]1.0
				[2]1.0
				[3]1.0
*mag*A	0 (0%)	0 (0%)	0 (0%)	N/A
*hly*A	0 (0%)	0 (0%)	0 (0%)	N/A
cnf-1	3 (9.4%)	0 (0%)	0 (0%)	[1]1.0
				[2]0.28

				[3]N/A
irp-2	12 (37.5%)	7 (70.0%)	10 (52.6%)	[1]0.14
				[2]0.38
				[3]0.45
irp-1	12 (37.5%)	7 (70.0%)	11 (57.9%)	[1]0.14
				[2]0.24
				[3]0.69
fyuA	10 (31.3%)	6 (60.0%)	12 (63.2%)	[1]0.14
				[2]0.04
				[3]1.0
entB	23 (71.9%)	9 (90%)	13 (68.4%)	[1]0.4
				[2]1.0
				[3]0.37
iutA	13 (40.6%)	1 (10.0%)	3 (15.8%)	[1]0.12
				[2]0.12
				[3]1.0
iroN	6 (18.8%)	4 (40.0%)	3 (15.8%)	[1]0.21
				[2]1.0
				[3]0.19

Supplementary Table 3. Comparison of the total number of bacterial virulence genes in *K. pneumoniae* isolates between RTx and non-RTx patients

Median (25-75 percentile)	**RTx (n=19)**	**Non-RTx (n=7)**	**p**
AB	6.5 (5-8)	6 (6-8)	0.89
lower UTI	7 (6-9)	8 (5.5-8.5)	0.97
upper UTI	7 (5-9)	8 (6-11)	0.19

Supplementary Table 4. Comparison of the total number of bacterial virulence genes in *K. pneumoniae* isolates according to type of UTI ([1]asymptomatic bacteriuria vs lower UTI, [2]asymptomatic bacteriuria vs upper UTI, [3]lower UTI vs upper UTI)

Median (25-75 percentile)	**AB**	**Lower UTI**	**Upper UTI**	**p**
RTx	6.5 (5-8)	7 (6-9)	7 (5-9)	[1]0.63
				[2]0.81
				[3]0.54
Non-RTx	6 (6-8)	8 (5.5-8.5)	8 (6-11)	[1]0.74
				[2]0.24
				[3]0.38

Supplementary Table 5. Comparison of bacterial virulence genes in *K. pneumoniae* isolates derived from cases of asymptomatic bacteriuria in RTx and non-RTx patients

Variable, n (%)	**RTx (n=32)**	**Non-RTx (n=17)**	**p**
fimH-1	27 (84.4%)	14 (82.4%)	1.0
kpn	29 (90.6%)	15 (88.2%)	1.0
*mrk*D	30 (93.8%)	14 (82.4%)	0.33
*ycf*M	24 (75.0%)	11 (64.7%)	0.52
uge	25 (78.1%)	12 (70.6%)	0.73
*rmp*A	3 (9.4%)	1 (5.9%)	1.0
*mag*A	0 (0%)	0 (0%)	N/A
*hly*A	0 (0%)	0 (0%)	N/A
cnf-1	3 (9.4%)	3 (17.7%)	0.41
irp-2	12 (37.5%)	10 (58.8%)	0.23
irp-1	12 (37.5%)	10 (58.8%)	0.23
fyuA	10 (31.3%)	7 (41.2%)	0.54
entB	**23 (71.9%)**	**6 (35.3%)**	**0.017**
iutA	13 (40.6%)	9 (52.9%)	0.55
iroN	6 (18.8%)	5 (29.4%)	0.48

Supplementary Table 6. Comparison of bacterial virulence genes in *K. pneumoniae* isolates derived from cases of lower urinary tract infections in RTx and non-RTx patients

Variable, n (%)	**RTx (n=10)**	**Non-RTx (n=12)**	**p**
fimH-1	7 (70.0%)	12 (100.0%)	0.08
kpn	6 (60.0%)	11 (91.7%)	0.14
*mrk*D	8 (80.0%)	11 (91.7%)	0.57
*ycf*M	8 (80.0%)	8 (66.7%)	0.65
uge	8 (80.0%)	10 (83.3%)	1.0
*rmp*A	0 (0%)	0 (0%)	N/A
*mag*A	0 (0%)	0 (0%)	N/A
*hly*A	0 (0%)	1 (8.3%)	1.0
cnf-1	0 (0%)	2 (16.7%)	0.48
irp-2	7 (70.0%)	7 (58.3%)	0.67
irp-1	7 (70.0%)	6 (50.0%)	0.41
fyuA	6 (60.0%)	5 (41.7%)	0.67
entB	9 (90.0%)	6 (50.0%)	0.07
iutA	1 (10.0%)	6 (50.0%)	0.07
iroN	**4 (40.0%)**	**0 (0%)**	**0.029**

Supplementary Table 7. Comparison of bacterial virulence genes in *K. pneumoniae* isolates derived from cases of upper urinary tract infections in RTx and non-RTx patients

Variable, n (%)	**RTx (n=19)**	**Non-RTx (n=7)**	**p**
fimH-1	16 (84.2%)	6 (85.7%)	1.0
kpn	11 (57.9%)	5 (71.4%)	0.67
*mrk*D	15 (79.0%)	7 (100%)	0.55
*ycf*M	10 (52.6%)	5 (71.4%)	0.66
uge	16 (84.2%)	5 (71.4%)	0.59
*rmp*A	1 (5.3%)	0 (0%)	1.0
*mag*A	0 (0%)	0 (0%)	N/A
*hly*A	0 (0%)	1 (14.3%)	0.27
cnf-1	0 (0%)	0 (0%)	N/A
irp-2	10 (52.6%)	5 (71.4%)	0.66
irp-1	11 (57.9%)	4 (57.1%)	1.0
fyuA	12 (63.1%)	6 (85.7%)	0.37
entB	13 (68.4%)	5 (71.4%)	1.0
iutA	**3 (15.8%)**	**6 (85.7%)**	**0.002**
iroN	3 (15.8%)	3 (42.9%)	0.29