Charaterization of multi-drug resistant *Klebsiella pneumoniae* isolates from urinary tract infected-women at Sylhet city, Bangladesh

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**ABSTRACT**

**Aim**: *Klebsiella pneumoniae* is considered to be one of the most frequent bacterial species associated with urinary tract infections (UTIs) and recurrent UTIs (RUTIs) worldwide. The present study aimed to comprehensively characterize *K. pneumoniae* isolates from women suffering from UTI and RUTI.

**Methodology and results**: A total of 15 clinical isolates, collected from different hospitals in Bangladesh, were tested for biochemical features, and amplified by PCR. Antibiogram assay was performed by disk-diffusion assay. Phylogenetic and functional features were analyzed using bioinformatics platform. XLSTAT was used for principal component analysis (PCA). PCR amplification using *Klebsiella* hemolysin gene (*khe*) confirmed the presence of *K. pneumoniae* in agarose gel with expected product size of 486 kb. Antibiogram assay revealed all *K. pneumoniae* isolates to be completely resistant to six out of ten relevant drugs namely ampicillin, cephradine, chloramphenicol, erythromycin, kanamycin and sulfamethoxazole used for treating UTIs in Bangladesh. Sequencing of 16s rRNA gene of clinically significant *K. pneumoniae* isolates showed a high level of sequence divergence between the isolates from UTI and RUTIs as well as functional features such as SNP variants and restriction sites.

**Conclusion, significance and impact of study**: We surmise that the results could be used as a pipeline for further research in the identification of *K. pneumoniae* associated with UTI and RUTIs, and treatment of infection.

**Keywords**: *Klebsiella pneumoniae*; *khe* gene; PCR; 16s rRNA sequencing; phylogenetic analysis

**INTRODUCTION**

Urinary tract infection (UTI) is a microbial infection, which is also the most commonly encountered hospital-acquired infection. It affects different parts of the urinary tract, with an occurrence both in males and females. However, women are more vulnerable to the infections owing to their reproductive physiology, i.e., shorter urethra in females allows a rapid transportation of bacteria from the anus to the bladder as compared to the male counterpart (Kolawole et al., 2009; Vasudevan, 2014). The literature reveals more than 60% of women to have a high risk of developing UTI in their lifetime; however, some of the infections remain asymptomatic for a prolonged period (Minardi et al., 2011). The term “RUTIs” was coined from the concept of re-isolating the bacteria responsible for causing infection during young age (Badr and Shaikh, 2013). In cases where bacteria exhibit tolerance to antibiotic treatment leading to their prolonged survival in the body, RUTIs can be lethal (Badr and Shaikh, 2013). A study revealed 27% of re-infection in women for the second time after 6 months of follow-up, whereas 2.7% of them reported having a third recurrent infection by the same bacteria (Hooton, 2001). A large group of bacteria is associated with UTI including strains from *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas sp.*; *Proteus sp.* and *Enterobacter sp.* Among these, *K. pneumoniae* was reported to be associated with 10 to 14% of global UTIs; in these cases, the pathogenicity and persistence were reported to be much higher than the bacterial counterpart *E. coli* (Kazemnia et al., 2014; Mahmudunnabi et al., 2018).

The most common problem that hinders the treatment of UTIs is drug resistance in clinical isolates; it poses a constant challenge to treat UTIs with commercially available treatments.

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available antibiotics. A multi-drug resistance (MDR) isolate displays complete resistance to three or more commercially available drugs that were reported to have increased sensitivity earlier (Nikaido, 2010). Insensitivity or resistance to antimicrobials has emerged as a common problem in the densely populated, low-income countries with poor hygiene and sanitation. The situation is aggravated owing to improper drug regime and frequent use of antibiotics to treat common infections (Mamunuddinabari et al., 2018). The ability of multidrug-resistant bacteria to combat attack by antimicrobial drugs leads to ineffective treatment, consequently resulting in the persistence and spreading of infections (Tanwar et al., 2014; Oliver et al., 2015).

Recent advancements in molecular methods have enabled rapid and comprehensive detection of various pathogens from clinical samples. Among numerous techniques, polymerase chain reaction (PCR) is most frequently used to identify bacteria (Barghouthi, 2011). The identification of targeted virulence factors and amplification of 16s RNA are the two most common practices in characterizing pathogens (López-banda et al., 2014; Srinivasan et al., 2015). The multi-functional and multi-factorial behavior of virulent genes makes them a key component in determining the pathogenicity of any isolate (Zhan and Zhu, 2017). The 16s rRNA gene of bacteria is associated with the advantage of providing large-scale sequence informatics on bacterial phylogeny and taxonomy. These act as low-resolution profiles in the traditional biochemical characterization and other plate-based methods (Janda and Abbott, 2007). Moreover, computer-aided bioinformatics tools and statistical packages make the critical analysis and interpretation easier (Allali et al., 2017).

In Bangladesh, the detection of K. pneumoniae-associated UTIs is still based on traditional phenotypic tests. These culture-based methods are time-consuming, require significant technical skills to interpret the data, often resulting in false-positive results (Yasmeen et al., 2015; Setu et al., 2016). The widespread use of molecular tools is extremely limited due to the lack of knowledge and technical skills. Since the selection of an appropriate treatment approach largely depends on finding the causative agent, therefore a comprehensive set of guidelines is necessary for precise identification of K. pneumoniae in Bangladesh.

**MATERIALS AND METHODS**

**Collection of samples**

A total of 15 clinical samples were collected from three different hospitals from Sylhet, Bangladesh, namely Sylhet MAG Osmani Medical College and Hospitals, Popular Hospitals and Diagnostic Centre, and Jalalabad Ragib Rabeya Medical College and Hospital, over a period of six months, from January to June 2017. The information of patients positive for UTI and K. pneumoniae were retrieved from the patient consent forms (Table 1). Then the samples were transported to the laboratory by maintaining cold condition (4 °C) for further analysis. The samples were initially cultured in chromogenic agar medium (Sigma-Aldrich, Germany), followed by and sub-cultured onto nutrient agar (NA) medium (Sigma-Aldrich, Germany) and incubated at 37 °C for 24 h. The overnight bacterial cultures were examined for biochemical characterization and genomic DNA extraction.

| Table 1: List of K. pneumoniae isolates with isolation history |
|---------------------------------|-----------|--------------|--------------------|-----------------------------|-------------------------|-----------|
| Id no. | Isolates | Age | Weight | Income | Physical status of the patient | Infection type | Hospitals |
|--------|----------|-----|--------|--------|-------------------------------|-----------------|-----------|
| 2667   | K11      | 38  | 58     | 300    | Secondary bacterial infection | First time      | 1         |
| 619    | K12      | 30  | 59     | 516    | Healthy                       | First time      | 1         |
| 702    | K13      | 35  | 62     | 480    | Secondary bacterial infection | First time      | 2         |
| 1775   | K14      | 28  | 55     | 600    | Fever, stomachache           | First time      | 1         |
| 1078   | K15      | 41  | 60     | 500    | Secondary bacterial infection | First time      | 3         |
| 937    | K16      | 35  | 58     | 500    | Fever, stomachache           | First time      | 3         |
| 235    | K17      | 38  | 58     | 360    | Malnutrition                 | First time      | 2         |
| 1140   | K18      | 65  | 65     | 480    | Secondary bacterial infection | First time      | 3         |
| 1235   | K19      | 30  | 61     | 300    | Fever, stomachache           | First time      | 3         |
| 785    | K20      | 32  | 65     | 360    | Pregnancy complications,     | Re-current      | 3         |
|        |          |     |        |        | Secondary bacterial infections|                 |           |
| 396    | K21      | 45  | 62     | 240    | Stomachache, flatulence       | First time      | 2         |
| 896    | K22      | 37  | 57     | 270    | Secondary bacterial infection | First time      | 2         |
| 905    | K23      | 68  | 59     | 600    | Malnutrition                 | First time      | 3         |
| 1526   | K24      | 65  | 62     | 580    | Secondary bacterial infection | First time      | 2         |
| 759    | K25      | 40  | 58     | 240    | Stomach pain, flatulence      | First time      | 3         |

*Income values are presented as thousand BDT. Hospitals: JRRMC = Jalalabad Ragib Rabeya Medical College and Hospitals, SMAGOMC = Sylhet MAG Osmani Medical College, PHDC = Popular Hospital and Diagnostic Centre.
Biochemical characterization of *K. pneumoniae* isolates

All isolates were subjected to several microbiological and biochemical tests according to the standard methods for identification of *K. pneumoniae* from clinical samples (Berger and Holt, 1994; Hansen et al., 2004). The isolates were assayed for Gram’s test, catalase, oxidase, methyl-red, Voges–Proskauer, citrate, urease, H₂S, and indole tests, motility, fermentation with glucose, sucrose, and maltose. Following the biochemical tests, positive isolates of *K. pneumoniae* were preserved and cultured.

Antibiogram profiling of *K. pneumoniae* isolates

Drug sensitivity assay with 10 commercially available antibiotics was conducted following disk-diffusion assay (Balouiri et al., 2016). The overnight bacterial culture (30 µL) was poured onto NA plates and spread uniformly with an L-shape glass rod. Then antibiotic discs were added aseptically onto the culture plate inside biosafety cabinet using sterile forceps and incubated at 37 °C overnight. Antibiotic disks (Oxoid, Thermo Scientific, USA) used in this study includedampicillin (AMP, 10 µg/disk), cephradine (CEP, 30 µg/disk), chloramphenicol (C, 30 µg/disk), ciprofloxacin (CP, 10 µg/disk), erythromycin (ERY, 15 µg/disk), kanamycin (K, 5 µg/disk), levofloxacin (LEV, 5 µg/disk), streptomycin (S, 10 µg/disk), sulfamethoxazole (SXT, 25 µg/disk), and tetracycline (T, 10 µg/disk). The zone of inhibition was measured according to previously described standard method following CLSI method (Rota et al., 2008).

Genomic DNA extraction and quantification

Genomic DNA was extracted using the ATP Genomic DNA Mini Kit (ATP Biotech, Inc., Taiwan) following manufacturer’s instructions. Proteinase K and RNase A were added to samples and incubated at 37 °C for 30 min to remove the impurities and enhance the quality of the extracted DNA. Extracted DNA was quantified using NanoDrop (Thermo Fisher Scientific, 2006c) by measuring the DNA–protein absorbance. The extracted DNA was then diluted accordingly to reach the final concentration of 30 ng/µL. Both stock and diluted DNA were then stored at −20 °C until use for PCR.

Amplification of *khe* and 16s rRNA gene

For *khe* PCR, the final master reaction volume was adjusted to 30 µL that contained 15 µL of 2 × master mixture (Fermentas, USA), 1.5 µL of each *khe* forward and reverse primers (5'-TGATTGCATTGCCACCTGG-3' and 5'-GGTCACCCCAAGCAGTCT-3'), 2 µL of template DNA and 10 µL of nuclease-free water (Jian-li et al., 2017). Finally, a total of 30 cycles of reaction was programmed in MultiGene Gradient Thermal Cycler (Labnet International Inc., USA) with an initial denaturation temperature of 94 °C for 4 min; denaturation step at 95 °C for 1.5 min, annealing at 58 °C for 1.5 min, an extension at 72 °C for 1.5 min, followed by a final extension step at 72 °C for 5 min and final storage at 4 °C. *K. pneumoniae* ATCC®33495 and nuclease-free water were used as positive and negative control for the validation of PCR amplification. For 16s rRNA, the universal 27F and 1492R universal primers (5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AGGAGGTATCCTACGGCA-3') were used with the same volume of master mixture and concentration of reagents described above while the PCR conditions were slightly modified (Srinivasan et al., 2015). The 32 cycles of reactions were programmed with the annealing at 52 °C for 30 s. All PCR products were separated by electrophoresis (Bio-Rad Laboratories Inc.) on 1.0% agarose gel containing 0.7% gel red. The amplified bands were visualized using the gel documentation system (FujiFilm LAS–4000 Image Analyzer, Boston Inc.).

Sequencing of 16s rRNA gene of *K. pneumoniae*

The amplified full-length PCR products of 16s rRNA of 1,465 bp were visualized on an agarose gel and purified using the PureLink PCR purification kit (Thermo Scientific, USA) according to the manufacturer’s instructions. The purified 16s rRNA PCR products were then sequenced from ‘1st BASE’ sequencing center, Malaysia. Raw sequences were analyzed using various software and web-based sequence analysis tools.

Analysis of sequence data and phylogenetic relationship

After sequencing, raw sequences were extracted, primarily edited, and assembled using BioEdit (v7.0.4) (Carlsbad, California, USA), Chromas (v2.01) (Technelysium Pty Ltd, Helensvale, QLD, Australia), and SeqMan Pro (v15.0) (Madison, Wisconsin, USA) tools (Montaz et al., 2018). DECIPHER (v9.20) (Sanger Institute, Hinxton, UK) was used to remove chimeras from the raw sequences. The edited DNA sequences in FASTA format were then run for homology in the NCBI nucleotide database using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast). Multiple alignment of the assembled sequences was performed in MEGA 7.0 (Pennsylvania State University, USA) using ClustalW and extracting the raw file in MEGA format. The phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap replicates in MEGA 7.0. The GenBank accession numbers of *E. coli* species indicate their parenthood to most similar sequences. Evolutionary divergence among sequences was calculated as p-distances after avoiding gaps and taking both transitions and transversions. SNP variants and restriction sites (REs) in the three *K. pneumoniae* sequences were analyzed in Geneious (v11.07) (Biomatters Ltd, Auckland, New Zealand) using default parameters.
Statistical analysis

To analyze the correlations among the set of observations (variables), principal component analysis (PCA) test was done using XLSTAT (Addinsoft, New York, USA) considering the number of antibiotic resistant as the primary component. The *K. pneumoniae* isolates were set as observation level while other variables (age, weight, hospitals and number of antibiotic resistance) were run for Pearson (r) correlation rank test in PCA. Correlations mono-plot created after avoiding of missing data in default parameters in separate sheet.

RESULTS

Biochemical characterization of *K. pneumoniae* isolates

All isolates were initially supplied as *K. pneumoniae* in the ESBL chromogenic medium. Morphological and biochemical characterization confirmed them to belong to *K. pneumoniae* species. All of them were negative for Gram’s test, indole, methyl-red, citrate, urease, and H₂S tests and motility; they tested positive for catalase, oxidase, and Voges–Proskauer tests. They could also successfully ferment glucose, sucrose, and maltose.

Drug resistance in *K. pneumoniae* isolates

In the present study, all *K. pneumoniae* isolates characterized from UTI exhibited a high percentage of drug resistance. These were completely resistant to ampicillin, cephalidine, chloramphenicol, erythromycin, kanamycin, and sulfamethoxazole. Isolates K13, K19, and K20 were completely resistant to all of the tested antibiotics in the present study. The in vitro disk diffusion assay revealed levofloxacin and ciprofloxacin to be two most effective drugs with 87% and 80% sensitivity, respectively. However, no drug was found to potentially inhibit all *K. pneumoniae* isolates (Figure 1).

Amplification of *khe* and 16s rRNA gene by PCR

All isolates (K11–K25) that were positive for *K. pneumoniae* in biochemical tests were also found to be positive for *khe* gene by PCR amplification showed the expected 486 bp band (Figure 2). Three isolates, namely K13, K19, and K20 that showed complete resistance to all antibiotics and were associated with other complications were selected for 16S rRNA gene amplification using 27F and 1492R universal sequencing primers. All isolates upon amplification displayed expected product size of 1,465 bp on an agarose gel.

Figure 1: Antibiotic resistance in *K. pneumoniae* isolates. The numerical values in right side indicate the number of resistant isolates.

Figure 2: Amplification of *khe* gene of *K. pneumoniae* by PCR. Lane 1 (L1) and lane 2 (L2) are 1kb DNA ladder (HyperLadder™, Bioline).

Sequence and data analysis

The present study found 99% homology of the sequenced isolates to clinical *K. pneumoniae* species in BLASTn at NCBI. Also, the study of phylogenetic tree revealed a close clustering of study isolates with strains of *K. pneumoniae* associated with UTIs (Figure 3). Evolutionary divergence data revealed fair distances within and among the study sequences (0.002–0.004), where the isolate *K. pneumoniae* K13 demonstrated a negligible detachment from *K. pneumoniae* subsp. *ozaenae* ATCC 11296 (0.001), a bacterium associated with UTI. Significant evolutionary divergence (0.237–0.285) was observed among other *K. pneumoniae* strains and two bacteria, namely *Actinobaculum massiliense* and *Staphylococcus saprophyticus*.

The SNP variant study in the 16S rRNA sequence of K20 straw found 28 SNPs, abundantly present all over the sequences (Figure 4). Restriction sites analysis found significant dissimilarities in the number and positions of the enzyme in strain K20. Analysis from position 1,078 to 1,096 revealed six digestion sites (Avall, Hpall, two Mspl, and two HaelII) in K20 compared to three in K13 and K19 (Hpall, Mspl, and HaelII). The study sequence is available in the GenBank databank of NCBI under the accession number of MG890348.1 (K13), MG890349 (K19), and MG890350 (K20). The PCA among variables found a correlations among antibiotic resistance, weight, and the sources of sample collection where the relationship was strong between drug resistance and weight compared to drug resistance and hospital sources. Whereas the number of drug resistance were independent of age and personal income. A distinct pattern of clustering for *K. pneumoniae* isolates also observed in the plot where characteristically similar isolates (K13, K19, K20, and K21) were clustered together. In the mono-plot, the axis F1 and F2 described 70.42% of variables, fair enough to make conclusive comments (Figure 5).
Figure 3: Evolutionary relationships of K. pneumoniae isolates associated with UTI. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 7. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

Figure 4: SNPs heat-map (first image) of the study K. pneumoniae isolates with other UTI associated K. pneumoniae strains. Out of 28, only two SNP variants (green box) were common in three study isolates. Predicted restriction (RE) sites (second image) found six sites in RUTIs associated K20 isolate (MG890350) compared three in two UTI causing isolates.

DISCUSSION

Humans are prone to a wide variety of infections caused by K. pneumoniae, including UTIs. The pathogenicity of K. pneumoniae associated with UTI is influenced by diverse factors, including population density, living conditions, proper hygiene practice, sanitation, diagnosis and treatment strategies, and physical and immune status of the patient (Zaffanello et al., 2010; Gupta and Trautner, 2013).
These factors are also responsible for causing RUTIs and secondary bacterial infections by *Chlamydia* and mycoplasmas (Matuszkiewicz-Rawińska et al., 2015). The observed uncharacterized secondary bacterial infections in half of (47%) the UTI patients in the present study signify the importance of these factors and the diagnostic limitations in Bangladesh. The spread of multi-drug resistant bacteria is attributed to misdiagnosis or late diagnosis of UTIs (Jian-li et al., 2017; Hawkey et al., 2018). The present study identified circulating *K. pneumoniae* from UTI and RUTIs in women using virulence factor-based PCR and 16s rRNA sequence analysis. The gene *khe* is reported to be a highly conserved virulent gene for *K. pneumoniae* that can be detected even at a very low concentration of genomic DNA (<100 copies) precisely and accurately (Clifford et al., 2012). All *K. pneumoniae* isolates tested positive for *khe* indicating this gene to be a better identification marker for the pathogenic features of bacteria. Here, we selected three of the most drug-resistant isolates that were also associated with complications for evolutionary informatics, and found substantial sequence divergence in the 16S rRNA data, especially in the isolates associated with RUTIs. These distances possibly arise from the differences in SNP variants and restriction sites. SNP variants, responsible for genetic differences in human, and have been found to be associated with an individual's drug response and susceptibility to a particular disease (Gamazon et al., 2010; Roden et al., 2012). The increasing number of SNP variants in a DNA sequence raises the probability of mutations within or near the regulatory genes directly involved in disease progression and reinfections through alteration of gene function (Li et al., 2014; Ma et al., 2015). The presence of two more SNP variants in RUTIs causing *K. pneumoniae*, therefore, could be responsible for other phylogenetic and functional differences as compared to other two isolates. Moreover, an analysis of restriction sites is significant in analyzing the multi-drug resistant strain as well as the recurring strains in clinical infections (Tahmasebi et al., 2012). Drug-specific receptors are usually more susceptible to point mutations, thereby leading to antibiotic resistance (Gorgani et al., 2010). Hence, we hypothesize that the modifications in RE sites from positions 1,078 to 1,096 possibly initiated from DNA sequence variants in *K. pneumoniae* associated with RUTIs and drug resistance. The present research could find no antibiotic with a potential to inhibit or kill experimental *K. pneumoniae* isolates. In addition, sensitivity to the drug was found to be specific to isolates and depended on patient's response and concomitant factors including type of infections, habitat, age, etc. Alarming drug resistance in *K. pneumoniae* associated with UTI become a major health concern in Bangladesh in recent years (Mahmudunnabi et al., 2018). The results indicate that analyzing the health status of the patient and the causative agent are equally important before selecting the appropriate treatment strategy for UTI and RUTIs. Finally, the correlations among antibiotic resistance with other variables in this study signify that a better health status, life style and living conditions could help to minimize the risk of UTI infection in women.

**CONCLUSION**

The current study describes a set of rapid, reliable, and accurate techniques for early and easier detection of *K. pneumoniae* in UTI and RUTIs. Besides, we also found a positive correlation among antimicrobial resistance, SNP variants, and RE sites of clinical *K. pneumoniae* isolates associated with UTI and RUTIs in women. However, the sample sizes was the major limitation of the study to make any conclusive comments based on research findings. Besides, this was a complete regional study where samples were collected from the Eastern part of Bangladesh. Therefore, it may not reflect the global scenario. Further long-term studies are required to analyze the drug resistance mechanism of UTI and RUTIs causing *K. pneumoniae* in Bangladesh.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest

**REFERENCES**

Allali, I., Arnold, J. W., Roach, J., Cadenas, M. B., Butz, N., Hassan, H. M., Koci, M., Ballou, A., Mendoza, M., Ali, R. and Azzarate-peril, M. A. (2017). A comparison of sequencing platforms and bioinformatics pipelines for compositional analysis of the gut microbiome. *BMC Microbiology* 17, 1-16.
Badr, A. A. and Shaikh, G. (2013). Recurrent urinary tract infections management in women. Sultan Qaboos University Medical Journal 13, 359-367.

Balouiri, M., Sadiki, M. and Ilnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 6, 71-79.

Bardavid, E. R. and Oren, A. (2012). Acid-shifted isoelectric point profiles of the proteins in a hypersaline microbial mat: An adaptation to life at high salt concentrations? Extremophiles 16, 787-792.

Barghouthi, S. A. (2011). A Universal method for the identification of bacteria based on general PCR primers. Indian Journal of Microbiology 51, 430-444.

Berger, D. H. and Holt, J. G. (1994). Bergey’s manual of determinative bacteriology. 9th Edition, Williams & Wilkins, Baltimore, Maryland, pp.1–887.

Clifford, R. J., Millilo, M., Prestwood, J., Quintero, R., Zurawski, D. V., Kwak, Y. I., Waterman, P. E., Lesho, E. P. and Mc-Gann, P. (2012). Detection of bacterial 16S rRNA and identification of four clinically important bacteria by Real-Time PCR. PLoS ONE 7, e46558.

Edwards, I. A., Elliott, A. G., Kavanagh, A. M., Zuegg, J., Blaskovich, M. A. T. and Cooper, M. A. (2016). Contribution of amphiaphopathy and hydrophobicity to the antimicrobial activity and cytotoxicity of β-hairpin peptides. ACS Infectious Diseases 2, 442-450.

Fedyukina, D. V., Jennaro, T. S. and Cavagnero, S. (2014). Charge segregation and low hydrophobicity are key features of ribosomal proteins from different organisms. The Journal of Biological Chemistry 289, 6740-6750.

Gamazon, E. R., Huang, R. S., Cox, N. J. and Dolan, M. E. (2010). Chemotherapeutic drug susceptibility associated SNPs are enriched in expression quantitative trait loci. Proceedings of the National Academy of Sciences 107, 9287-9292.

Gorgani, N., Ahlbrand, S., Patterson, A. and Pourmand, N. (2010). Detection of point mutations associated with antibiotic resistance in Pseudomonas aeruginosa. International Journal of Antimicrobial Agents 34, 414-418.

Gupta, K. and Trautner, B. W. (2013). Diagnosis and management of recurrent urinary tract infections in non-pregnant women. British Medical Journal 346, f3140.

Hansen, D. S., Aucken, H. M., Abiola, T. and Poddusch, R. (2004). Recommended test panel for differentiation of Klebsiella species on the basis of a triliteral interlaboratory evaluation of 18 biochemical tests. Journal of Clinical Microbiology 42, 3665-3669.

Hawkey, P. M., Warren, R. E., Livermore, D. M., McNulty, C. A. M., Enoch, D. A., Otter, J. A. and Wilson, A. P. R. (2018). Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/ Healthcare Infection Society/British Infection Association Joint Working Party. Journal of Antimicrobial Chemotherapy 73, iii2-iii78.

Hooton, T. M. (2001). Recurrent urinary tract infection in women. International Journal of Antimicrobial Agents 17, 29-26.

Janda, J. M. and Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. Journal of Clinical Microbiology 45, 2761-2764.

Jian-li, W., Yuan-yuan, S., Shou-yu, G., Fei-lei, D. and Jia-yu, Y. (2017). Serotype and virulence genes of Klebsiella pneumoniae isolated from mink and its pathogenesis in mice and mink. Scientific Reports 7, 1-7.

Kazemnia, A., Ahmad, M. and Dilmaghani, M. (2014). Antibiotic resistance pattern of different Escherichia coli phylogenetic groups isolated from human urinary tract infection and avian colibacillosis. Iranian Biomedical Journal 18, 219-224.

Kiraga, J., Mackiewicz, P., Mackiewicz, D., Kowalcuz, M., Polak, N., Smolarczyk, K., Dudek, M. R. and Cebrat, S. (2007). The relationships between the isoelectric point and length of proteins, taxonomy and ecology of organisms. BMC Genomics 16, 1-16.

Kolawole, A. S., Kolawole, O. M., Kandaki-Oluwemi, Y., Babutunde, S. K., Durowade, K. A. and Kolawole, C. F. (2009). Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. International Journal of Medicine and Medical Sciences 1, 163-167.

Li, G., Pan, T., Guo, D. and Li, L. (2014). Regulatory variants and disease: the E-cadherin-160C/a SNP as an Example. Molecular Biology International 2014, ID967565.

Mahmudunnabi, G., Majlish, A. N. K., Momtaz, F., Izadi, A., Mahmud, S. and Sheli, A. (2013). Identification of virulence factors genes in Escherichia coli isolates from women with Urinary Tract Infection in Mexico. BMC Genomics 14, 268.

Muzzonigro, G., Cantoro, A. and Minardi, M. (2012). Molecular detection and PCR-RFLP analysis using PstI and AluI of multidrug resistant Klebsiella pneumoniae causing urinary tract infection in women in the eastern part of Bangladesh. Journal of Genetic Engineering and Biotechnology 16, 77-82.

Mahmudunnabi, G., Majlish, A. N. K., Momtaz, F., Izadi, A., Mahmud, S. and Sheli, A. (2013). Identification of virulence factors genes in Escherichia coli isolates from women with Urinary Tract Infection in Mexico. BMC Genomics 14, 268.

Muzzonigro, G., Cantoro, A. and Minardi, M. (2012). Molecular detection and PCR-RFLP analysis using PstI and AluI of multidrug resistant Klebsiella pneumoniae causing urinary tract infection in women in the eastern part of Bangladesh. Journal of Genetic Engineering and Biotechnology 16, 77-82.

Minardi, D., D’Anzeo, G., Cantoro, D., Conti, A. and Muzzonigro, G. (2011). Urinary tract infections in...
women: etiology and treatment options. *International Journal of General Medicine* 4, 333-343.

Momtaz, F., Ali, M. H., Hossain, M. N., Foysal, M. J., Sumiya, M. K. and Islam, K. (2018). Characterisation of multidrug-resistant *Alcaligenes faecalis* strain AF1 isolated from patient of RUTIs: A study from Bangladesh. *Journal of Clinical and Diagnostic Research 12*, KC01-KC04.

Nikaido, H. (2010). Multidrug resistance in bacteria. *Annual Review in Biochemistry* 78, 119-146.

Oliver, W., Kempf, V. A., Brandt, C., Zeuzem, S., Piiper, A. and Kronenberger, B. (2015). Colonisation with multidrug-resistant bacteria is associated with increased mortality in patients with cirrhosis. *Gut* 64, 1183-1184.

Roden, D. M., Wilke, R. A., Kroemer, H. K. and Stein, M. C. (2012). Pharmacogenomics: The genetics of variable drug responses. *Circulation* 123, 1661-1670.

Rota, M. C., Herrera, A., Martinez, R. M., Sotomayor, J. A. and Jordán, M. J. (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control* 19, 681-687.

Setu, S. K., Naser, A., Sattar, I., Saleh, A. A., Roy, C. K. and Ahmed, M. (2016). Study of bacterial pathogens in urinary tract infection and their antibiotic resistance profile in a tertiary care hospital of Bangladesh. *Bangladesh Journal of Medical Microbiology* 10, 22-26.

Srinivasan, R., Karaoz, U., Volegov, M., MacKichan, J., Kato-Maeda, M., Miller, S., Nadarajan, R., Brodie, E. L. and Lynch, S. V. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS ONE* 10, e4114.

Tahmasebi, P., Farnia, P., Sheikholeslami, F. and Velayati, A. (2012). Rapid identification of extensively and extremely drug resistant tuberculosis from multidrug resistant strains; using PCR-RFLP and PCR-SSCP. *Iranian Journal of Microbiology* 4, 165-170.

Tanwar, J., Das, S., Fatima, Z. and Hameed, S. (2014). Multidrug resistance: An emerging crisis. *Interdisciplinary Perspectives on Infectious Diseases* 2014, 1-7.

Vasudevan, R. (2014). Urinary tract infection: An overview of the infection and the associated risk factors. *Journal of Microbiology and Experimentation* 1, 1-15.

Yasmeen, B. H. N., Islam, S., Uddin, M. M. and Jahan, R. (2015). Prevalence of urinary tract infection, its causative agents and antibiotic sensitivity pattern: A study in Northern International Medical College Hospital, Dhaka. *Northern International Medical College Journal 7*, 105-109.

Zaffanello, M., Malerba, G., Cataldi, L., Antoniazzi, F., Franchini, M., Monti, E. and Fanos, V. (2010). Genetic risk for recurrent urinary tract infections in humans: a systematic review. *Journal of Biomedicine and Biotechnology* 2010, ID321082.

Zhan, X., Zhu, Q. (2017). Molecular evolution of virulence genes and non-virulence genes in clinical, natural and artificial environmental *Legionella pneumophila* isolates. *PeerJ* 5, e4114, 1-21.