Readers Insight
Publisher of open access books and journals
PJMLS OPEN ACCESS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/syr1zh46	Copyright © All rights are reserved by Corresponding Author
Vol. 8 No. 2, 2025: pp. 393-398	
www.readersinsight.net/pjmls	**Revised:** June 18 , 2025 **Accepted:** June 25, 2025
Submission: May 03, 2025	**Published Online:** June 30, 2025

MOLECULAR SURVEILLANCE OF 16S RRNA METHYLASE GENES IN AMINOGLYCOSIDE-RESISTANT PROTEUS MIRABILIS

Iqra Sarwar[1], Ayaz Ali Panhwar[2], Hina Tahir[3], Zubair Akram[4], Mahnoor Javed[5], Faheem Akhtar[6], Tahir Hasan[7], Amanullah Maree[8], Abdul Wahab[1]*

[1]**Institute of Microbiology,** Government College University, Faisalabad, Pakistan
[2]**Department of Veterinary Pathology,** Sindh Agriculture University, Tandojam, Pakistan
[3]**Department of Biochemistry,** Arid Agriculture University, Rawalpindi, Pakistan
[4]**Department of Biochemistry,** University of Agriculture, Faisalabad, Pakistan
[5]**Department of Zoology,** University of Sargodha, Sarggodha, Pakistan
[6]**Institute of Zoology,** Bahauddin Zakariya University, Multan, Pakistan
[7]**Centre of Biotechnology and Microbiology,** University of Peshawar, Peshawar, Pakistan
[8]**Department of Plant Breeding and Genetics,** Sindh Agriculture University, TandoJam, Pakistan

***Correspondence author:** Abdul Wahab. E. mail: wahabsheikh141@gmail.com

Abstract

Proteus mirabilis is a Gram-negative, rod-shaped bacterium commonly associated with catheter-associated urinary tract infections (CAUTIs), largely due to its swarming motility and urease activity. These infections are often preceded by urolithiasis, including bladder and kidney stone formation. Aminoglycosides have traditionally been effective in treating a broad range of bacterial infections by binding to the A-site of 16S rRNA in the 30S ribosomal subunit, thereby inhibiting protein synthesis. However, resistance to aminoglycosides has emerged due to the acquisition of aminoglycoside-modifying enzymes such as acetyltransferases, phosphotransferases, and nucleotidyltransferases. More concerning is the recent global dissemination of plasmid-mediated 16S rRNA methyltransferase genes—rmtA, rmtB, and rmtC, which confer high-level resistance and pose a serious clinical threat. In this study, P. mirabilis isolates were molecularly confirmed through amplification of the ureC gene. Antimicrobial susceptibility to aminoglycosides was assessed using the Kirby-Bauer disc diffusion method and broth microdilution assays. The presence of 16S rRNA methylase genes in resistant isolates was subsequently detected via PCR.

Keywords: *16S rRNA methylases, Aminoglycoside resistance, CAUTIs, PCR, Proteus mirabilis, rmtA, rmtB, rmtC, Swarming motility, UreC gene, Urease activity*

INTRODUCTION

Proteus mirabilis, first described by the German scientist Gustav Hauser, is a Gram-negative, rod-shaped bacterium known for its characteristic swarming motility and bullseye colony formation on agar plates (1). A member of the class *Gammaproteobacteria*, order *Enterobacterales*, and previously of the *Enterobacteriaceae* family, *P. mirabilis* has now been reclassified under the *Morganellaceae* family based on phylogenomic analysis (2). The genus *Proteus* includes five recognized species: *P. mirabilis*, *P. hauseri*, *P. penneri*, *P. myxofaciens*, and *P. vulgaris* (3). Among these, *P. mirabilis* is a key uropathogen, possessing various virulence factors such as urease, fimbriae, adhesins, proteases, siderophores, and the ability to form biofilms (4). Its flagellar motility and secretion of extracellular polysaccharides contribute to its colonization on medical devices (5).

P. mirabilis inhabits diverse environments, including water, soil, and the gastrointestinal tract of humans and animals (6). It is a leading cause of complicated urinary tract infections (UTIs) and catheter-associated UTIs (CAUTIs), particularly in patients with prolonged catheterization and underlying conditions (7-9). The pathogenicity of *P. mirabilis* is enhanced by its urease activity, which hydrolyzes urea into ammonia, increasing urinary pH and promoting crystal and stone formation (10, 11). These urinary stones protect the bacteria from antibiotics and host immune responses, contributing to treatment failure and recurrence (12). In vulnerable populations such as the elderly and immunocompromised, *P. mirabilis* can cause systemic infections including bacteremia, sepsis, and meningitis (13, 14).

Aminoglycosides are widely used antibiotics that inhibit bacterial protein synthesis by binding to the A-site of 16S rRNA within the 30S ribosomal subunit (15). Despite their broad-spectrum activity, rising resistance due to aminoglycoside-modifying enzymes and target site modification has become a major clinical concern (16, 17). One important resistance mechanism involves plasmid-mediated 16S rRNA methyltransferases—enzymes such as ArmA, RmtA, RmtB, RmtC, and RmtD—that methylate specific nucleotides in the ribosome, preventing aminoglycoside binding (18, 19).

Several methylase genes, particularly *armA* and *rmtB*, have been reported worldwide, especially in multidrug-resistant *Enterobacteriaceae* (20, 21). In *P. mirabilis*, the emergence of these methylases poses a growing threat to the efficacy of aminoglycosides. The first 16S rRNA methylase gene, *rmtA*, was reported in *Pseudomonas aeruginosa* strain AR-2 in Japan (22), followed by the identification of *rmtC* and other variants in different bacterial hosts (23).

Given the clinical relevance of *P. mirabilis* and the critical role of aminoglycoside resistance mechanisms in therapy failure, this study was conducted with the objectives; confirmation of *Proteus mirabilis* using *ureC* gene amplification, screening of *P. mirabilis* isolates for resistance to aminoglycosides using Kirby-Bauer disc diffusion and broth microdilution assays and detection of 16S rRNA methylase genes (*armA*, *rmtA*, *rmtB*, *rmtC*, and *rmtD*) in aminoglycoside-resistant isolates.

MATERIALS AND METHODS

STUDY DESIGN AND ETHICAL APPROVAL

This study involved *P. mirabilis* isolates from urine samples of UTI patients. Informed consent was obtained from participants, and ethical approval was granted by the Ethical Review Committee of Government College University, Faisalabad.

SAMPLE COLLECTION AND PROCESSING

Midstream clean-catch urine samples were collected using sterile containers from 100 patients visiting public and private hospitals in Faisalabad. Samples were transported at 4°C under sterile conditions to the Postgraduate Research Laboratory, Department of Microbiology, GCUF (31). Samples were inoculated on MacConkey, CLED, and blood agar for primary isolation and characterization of *P. mirabilis*. Swarming motility was observed on blood agar, while colony morphology and lactose fermentation were assessed on CLED and MacConkey agar.

BIOCHEMICAL IDENTIFICATION AND GRAM STAINING

Isolates were subjected to the following biochemical tests such as Urease Test (Stuart's broth), Catalase Test, Methyl Red and Voges–Proskauer Tests, Triple Sugar Iron (TSI) Test, Indole Test (distinguishes *P. mirabilis* from indole-positive *Proteus* species) and Oxidase Test. Further confirmation was performed using API 20E strips following the manufacturer's guidelines (BioMérieux, France).

Gram staining was performed using standard procedures, as adopted Tanvir et al., to observe cell morphology and staining reaction under a microscope (24).

DNA EXTRACTION AND GEL ELECTROPHORESIS

Genomic DNA was extracted using the boiling method and FavorPrep DNA extraction kit (Favorgen Biotech Corp., Taiwan). DNA was quantified by spectrophotometry at 260/280 nm using the standard formula: DNA Conc. (µg/ml) = OD260 × Dilution Factor × 50

Extracted DNA was analyzed on a 1% agarose gel stained with ethidium bromide and visualized using a UV transilluminator (25).

MOLECULAR CONFIRMATION VIA PCR

P. mirabilis was confirmed through amplification of the urease gene (*ureC*) using the following primers (26) Takeuchi et al., 1996):

ureC1: 5'-CCGGAACAGAAGTTGTCGCTGGA-3'

ureC2: 5'-GGGCTCTCCTTACCGACTTGATC-3'

ANTIBIOTIC SUSCEPTIBILITY TESTING

Aminoglycoside resistance was determined using Kirby-Bauer disc diffusion method on Mueller Hinton Agar (CLSI, 2015) and Microbroth dilution assay to determine MICs as per CLSI guidelines. A 0.5 McFarland standard was used for inoculum standardization. Zones of inhibition were measured in millimeters and interpreted following CLSI breakpoints.

DETECTION OF 16S RRNA METHYLASE GENES

PCR was performed using specific primers for *armA*, *rmtA*, *rmtB*, *rmtC*, and *rmtD* to detect the presence of 16S methyltransferase genes responsible for aminoglycoside resistance.

RESULTS

A total of 94 urine specimens were collected from catheterized patients and screened for bacterial growth. Various culture media including CLED agar, MacConkey agar, and blood agar were utilized for initial cultivation. Only isolates morphologically consistent with *Proteus* species were selected for further characterization.

IDENTIFICATION OF *PROTEUS* ISOLATES

On CLED agar, *Proteus* isolates produced translucent blue colonies, indicating lactose non-fermentation. On MacConkey agar, they formed pale, non-lactose-fermenting colonies. Swarming motility, a hallmark of *P. mirabilis*, was confirmed on blood agar plates. Based on these phenotypic characteristics, 94 isolates were presumed to be *Proteus* spp.

All isolates showed optimal growth at 37°C for 24 hours under aerobic conditions on CLED, MacConkey, and blood agar. *P. mirabilis* is a facultative anaerobe and can grow in both aerobic and anaerobic environments (33). All isolates were Gram-negative straight rods, as evidenced by pink staining under oil immersion microscopy.

BIOCHEMICAL CHARACTERIZATION

Urease Test: All isolates tested positive, consistent with the production of urease by *P. mirabilis*, which hydrolyzes urea into ammonia and carbon dioxide, increasing pH and changing the medium color to pink (28).

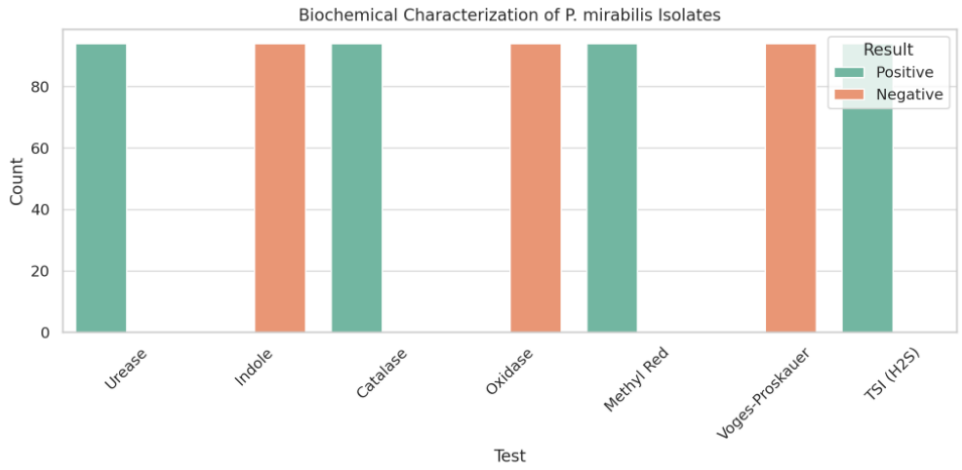

Fig. 1. All isolates were positive for urease, catalase, methyl red, TSI (H_2S) and negative for indole, oxidase, and Voges-Proskauer tests.

Indole Test: All isolates were indole-negative, differentiating them from *P. vulgaris*, which is indole-positive.
Catalase Test: All isolates were catalase-positive, capable of degrading hydrogen peroxide.
Oxidase Test: All isolates tested negative for oxidase activity.
MR-VP Test: All isolates were methyl red positive (indicating mixed acid fermentation) and Voges-Proskauer negative.

Triple Sugar Iron (TSI) Test: All isolates produced hydrogen sulfide (H_2S), turned slants red and butts yellow, with gas production noted, resulting in black precipitates in the medium (Fig. 1).

SPECIES CONFIRMATION AND GENDER DISTRIBUTION OF ISOLATES

Further identification using the API® identification system confirmed all 94 isolates as *P. mirabilis*. Out of the 94 confirmed *P. mirabilis* isolates, 55 (59%) were recovered from female patients and 39 (41%) from male patients, suggesting a higher prevalence in females, likely due to anatomical susceptibility to urinary tract infections (Fig. 2a).

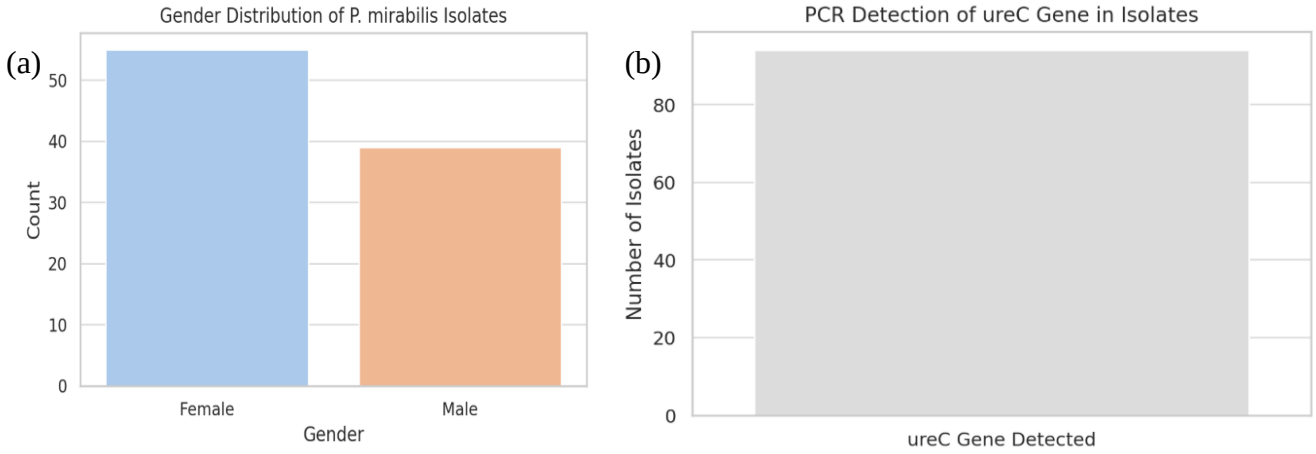

Fig. 2 (a). Gender distribution of *P. mirabilis* Isolates. A higher number of isolates were found in females (59%) than in males (41%); **(b).** PCR detection of ureC gene; all 94 isolates tested positive, indicating the presence of the ureC gene in every case

DNA EXTRACTION AND PCR AMPLIFICATION OF UREC GENE

DNA extraction using a commercial kit was confirmed through Nanodrop spectrophotometry and gel electrophoresis. Consistent and dark bands were visualized on 1% agarose gel, indicating high-quality genomic DNA. PCR amplification showed that all isolates harbored the *ureC* gene, producing ~533 bp amplicons. This gene encodes urease C subunit, crucial for urea hydrolysis in *P. mirabilis* (Fig. 2b).

ANTIBIOGRAM PROFILING

Antimicrobial susceptibility testing using both Kirby-Bauer Disc Diffusion and Microbroth Dilution methods revealed high resistance to multiple aminoglycoside antibiotics. A significant proportion of isolates displayed resistance patterns suggestive of 16S rRNA methyltransferase production.

DISCUSSION

The isolation and identification of 94 *Proteus mirabilis* strains from catheterized patients reinforce its role as a predominant causative agent in catheter-associated urinary tract infections (CAUTIs). The bacterium's swarming motility, urease activity, and ability to form biofilms on catheter surfaces contribute to its pathogenicity (27). The biochemical profile obtained aligns with standard descriptions of *P. mirabilis*—urease-positive, indole-negative, oxidase-negative, and capable of producing H_2S. The uniform presence of the *ureC* gene across all isolates underscores its role in urease biosynthesis and virulence (28).

The antimicrobial resistance data is particularly concerning. The widespread resistance to aminoglycosides such as gentamicin and amikacin indicates the probable dissemination of 16S rRNA methyltransferase genes (e.g., *armA*, *rmtB*), which confer high-level resistance by methylating the aminoglycoside-binding site on the 16S rRNA (17, 29). These enzymes have been increasingly reported in *Proteus* species and other Enterobacteriaceae, contributing to treatment failures. The predominance of isolates from female patients is consistent with higher rates of UTIs among women, attributed to shorter urethral length and proximity to the anus, facilitating ascending infections (30). The consistency and clarity of DNA extracted allowed successful amplification of target genes, establishing a robust foundation for downstream molecular analyses, including screening for resistance genes.

The detection of multiple resistance determinants in the isolates emphasizes the need for continuous surveillance of antimicrobial susceptibility patterns in hospital environments. Periodic monitoring can help in timely revision of empirical treatment guidelines and in the implementation of targeted infection control strategies to prevent the spread of multidrug-resistant strains (31).

Additionally, the strong biofilm-forming capacity observed in several isolates poses a challenge for eradication using conventional antibiotics, as biofilm-associated cells often exhibit significantly higher tolerance to antimicrobial agents and host immune defenses (32). Future studies could focus on evaluating anti-biofilm agents or catheter materials with anti-adherence properties to reduce the risk of persistent infections.

Finally, molecular epidemiology tools such as pulsed-field gel electrophoresis (PFGE) or whole-genome sequencing (WGS) could be employed to trace the clonal relationships among isolates, identify transmission pathways, and detect emerging high-risk clones. Such approaches could provide a more comprehensive understanding of the genetic basis of virulence and resistance in P. mirabilis, thereby informing both therapeutic and preventive measures (33).

CONCLUSION

This study confirms the high prevalence of aminoglycoside-resistant *Proteus mirabilis* isolates in catheterized patients, with biochemical and molecular characterization affirming the identity and resistance mechanisms of the strains. The uniform detection of the *ureC* gene and resistance profiles supports the hypothesis that methylation-based resistance mechanisms, such as 16S rRNA methylases, are prevalent in clinical *P. mirabilis* isolates. Enhanced surveillance and strict antibiotic stewardship are crucial to curb the spread of multidrug-resistant pathogens in healthcare settings.

Authors' contribution:

IS and AAP Research work, writing initial manuscript; HT and ZA data acquisition; MJ, FA and TH Data analysis, statistical analysis; AM Editing of manuscript; AW Conceptualization & supervision.

References:

1. Schwarzhoff RH, Williams RP. Formation of swarming growth patterns by Proteus mirabilis. Journal of Bacteriology. 1978;134(1):10–16.
2. Adeolu M, Alnajar S, Naushad S, Gupta RS. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': Proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology. 2016;66(12): 5575–5599.
3. O'Hara CM, Brenner FW, Miller JM.Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clinical Microbiology Reviews. 2000;13(4):534–546.
4. Schaffer JN, Pearson MM. Proteus mirabilis and urinary tract infections. Microbiology Spectrum. 2015; 3(5).
5. Foris LA, Snowden J. Proteus mirabilis. In StatPearls. StatPearls Publishing. 2017.
6. Armbruster CE, Mobley HLT. Merging mythology and morphology: The multifaceted lifestyle of Proteus mirabilis. Nat Rev Microbiol. 2012;10(11):743-54.
7. Warren JW, Platt R, Thomas RJ, Rosener B, Kass EH. Antibiotic treatment of bacteriuria in patients with indwelling catheters: A clinical trial. Ann Intern Med. 1982;97(5):713-8.
8. Kim BN, Woo JH, Kim MN, Ryu J, Kim YS. Clinical significance and outcomes of bacteremia caused by Proteus species. Korean J Intern Med. 2003;18(2):87-93.
9. Hung CH, Lin CH, Lin HY. Proteus mirabilis bacteremia: Risk factors and clinical characteristics. South Med J. 2007;100(8):783-7.
10. Stickler DJ, Morgan SD, Winters C. Clinical challenge: biofilms and bladder stones. Clin Infect Dis. 2006;43(5):652-7.

11. Coker C, Poore CA, Li X, Mobley HLT. Pathogenesis of urinary tract infection. Curr Opin Microbiol. 2000;3(1):65-70.
12. Foxman B, Brown P. Epidemiology of urinary tract infections: Transmission and risk factors, incidence, and costs. Infect Dis Clin North Am. 2003;17(2):227-41.
13. Mansy SS. Proteus mirabilis meningitis in an adult. J Infect. 2001;42(2):130-1.
14. Watanakunakorn C, Perni SC. Proteus mirabilis bacteremia: A review of 176 cases at a community teaching hospital. South Med J. 1994;87(2):258-60.
15. Kotra LP, Haddad J, Mobashery S. Aminoglycosides: Perspectives on mechanisms of action and resistance and strategies to counter resistance. Antimicrob Agents Chemother. 2000;44(12):3249-56.
16. Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev. 1993;57(1):138-63.
17. Wachino J, Arakawa Y. Exogenously acquired 16S rRNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: An update. Drug Resist Updat. 2012;15(3):133-48.
18. Galimand M, Sabtcheva S, Courvalin P, Lambert T. Worldwide dissemination of the 16S rRNA methylase armA gene among Gram-negative rods. Emerg Infect Dis. 2003;9(6):810-3.
19. Doi Y, Arakawa Y. 16S ribosomal RNA methylation: Emerging resistance mechanism against aminoglycosides. Clin Infect Dis. 2007;45(1):88-94.
20. Lee K, Lee WG, Uh Y, Ha GY, Cho J, Chong Y. VIM- and IMP-type metallo-β-lactamase-producing Pseudomonas spp. and Acinetobacter spp. in Korean hospitals. Emerg Infect Dis. 2006;12(8):1468-71.
21. Yamane K, Wachino J, Suzuki S, Kimura K, Shibata N, Kato H. New plasmid-mediated 16S rRNA methyltransferase, RmtC, found in a Proteus mirabilis clinical strain isolated in Japan. Antimicrob Agents Chemother. 2007;51(12):4401-4.
22. Yokoyama K, Doi Y, Yamane K, Kurokawa H, Shibata N, Shibayama K. Acquisition of 16S rRNA methylase gene rmtA in Pseudomonas aeruginosa. Lancet. 2003;362(9391):1888-93.
23. Yu Y, Zhou H, Xu X, Zhang J, Chen Y, Zhou Y, et al. Prevalence of 16S rRNA methylase genes among panaminoglycoside-resistant isolates of Gram-negative bacteria. J Clin Microbiol. 2007;45(3):881-5.
24. Tanvir F, Ahmad FJ, Khalid N, Javed MT. Characterization and antibiotic susceptibility pattern of bacterial pathogens from urinary tract infection in Gujranwala, Pakistan. Biologia (Pakistan). 2012;58(1–2):109-14.
25. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press; 2001.
26. Takeuchi K, Kato Y, Ohno T. Detection of Proteus mirabilis using urease gene-specific primers. Microbiol Immunol. 1996;40(11):827-31.
27. Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 2008;21(1):26-59.
28. Mobley HLT, Chippendale GR, Swihart KG. Cytotoxicity of Proteus mirabilis hemolysin to human renal cell lines. Infect Immun. 1995;63(1):102-7.
29. Wachino J, Yamane K, Kimura K, Shibayama K, Suzuki S, Ike Y. Novel plasmid-mediated 16S rRNA methylase, RmtD, found in a Proteus mirabilis isolate from Brazil. Antimicrob Agents Chemother. 2007;51(4):1380-2.
30. Foxman B. Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. Dis Mon. 2002;49(2):53-70.
31. Bonacorsi S, Clermont O, Houdouin V, Cordevant C, Brahimi N, Bingen E. Molecular analysis and experimental virulence of French human Escherichia coli strains producing CTX-M beta-lactamases. J Antimicrob Chemother. 2006;58(4):744-9.
32. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 25th Informational Supplement (M100-S25). Clinical and Laboratory Standards Institute. 2015.
33. Drzewiecka D. Significance and roles of Proteus spp. bacteria in natural environments. Microb Ecol. 2016;72(4):741-58.