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Brief communication (Original)

Antibiotic resistance, multidrug resistance and enterobacterial repetitive intergenic consensus polymerase chain reaction profiles of clinically important *Klebsiella* species

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Background: *Klebsiella* species are important opportunistic pathogens causing a variety of infections, especially in hospitalized and immunocompromised patients.
Objectives: To investigate the clinical prevalence of five different *Klebsiella* species (*K. pneumoniae*, *K. ornithinolytica*, *K. oxytoca*, *K. terrigena*, and *K. rhinoscleromatis*) including antibiotic resistance profiles using six different antibiotics and combinations (trimethoprim-sulfamethoxazole, ampicillin-sulbactam, imipenem, piperacillin-tazobactam, ciprofloxacin, ceftizoxime).
Methods: Resistance of *Klebsiella* spp. including multidrug resistant (MDR) strains was determined by using a Kirby–Bauer disk diffusion method and genotypical analysis was performed by enterobacterial repetitive intergenic consensus (ERIC) polymerase chain reaction (PCR).
Results: Urine samples and the urology service unit were the most common sources of *K. ornithinolytica*, *K. pneumoniae*, and *K. terrigena* strains. The greatest drug resistance was observed against trimethoprim-sulfamethoxazole (88%), the least resistance was observed against imipenem (12%). Apart from these, 11 different antibiotypes were generated and antibiotype AI (resistant only to trimethoprim-sulfamethoxazole) was the most frequently observed (40%). MDR profiles of *Klebsiella* spp. were also investigated and 25% of all *Klebsiella* spp. strains were found to be MDR; and 65% of these were isolated from urine samples. MDR strains were mostly found to be *K. ornithinolytica* (35%) followed by *K. pneumoniae* (29%). Genotyping was performed by using ERIC PCR and *Klebsiella* spp. strains were grouped in 23 genotypes with a similarity coefficient of 70%.
Conclusions: Antibiotyping and antibiotype profiles may provide valuable information for hospitalized patients that could identify problem spots and allow evidence-based provision of preventive measures against nosocomial emergence of infections with new MDR strains.

Keywords: Antibiotic resistance, ERIC PCR, *Klebsiella* spp., multidrug resistance

Reservoirs of drug resistant bacterial genomes and extrachromosomal DNA segments are a growing problem and cause emergence of new multidrug resistant (MDR) strains [1]. Antibiotic resistance of *Klebsiella* infections are causing increasing morbidity and mortality, and an increase in health care costs worldwide.

In epidemiological research, not only phenotypical analysis, but also genotypical analysis is conducted by using various molecular typing methods such as plasmid profiling, ribotyping, and polymerase chain reaction (PCR) to find genetic relationships between clinically important bacterial species [2, 3]. Our present study focused on prevalence of five *Klebsiella* spp. (*K. pneumoniae*, *K. ornithinolytica*, *K. oxytoca*, *K. terrigena*, and *K. rhinoscleromatis*) found in clinical materials. We examined antibiotic resistance and multidrug resistant strains and determined enterobacterial repetitive intergenic consensus (ERIC) PCR profiles for all *Klebsiella* spp.

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Materials and methods

Bacterial strains

We used 5 different *Klebsiella* spp. (*K. ornithinolytica*, *K. pneumoniae*, *K. oxytoca*, *K. terrigena*, and *K. rhinoscleromatis*) that had been isolated in a previous study [4].

Antibiotic resistance testing and antibiotyping

The antibiotic resistance of *Klebsiella* species to six different antibiotics and combinations (SXT: trimethoprim-sulfamethoxazole (1.25/23.75 μg), SAM: ampicillin-sulbactam (10/10 μg), IPM: imipenem (10 μg), TZP: piperacillin tazobactam (100/10 μg), CIP: ciprofloxacin (5 μg), CZ: ceftizoxime (30 g)) were assessed using a Kirby–Bauer disc diffusion method. *Klebsiella* spp. that showed the same antibiotic resistance pattern, were grouped in the same antibiotype. MDR was defined as being resistant to at least three or more of antimicrobial classes [5].

Genomic DNA Extraction

The genomic DNA of *Klebsiella* spp. was extracted from bacterial cultures by using a bacterial DNA extraction kit (BioBasic, East Markham, Ontario, Canada) and isolated DNA was stored at –20°C.

Fingerprinting by enterobacterial repetitive intergenic consensus polymerase chain reaction

We determined the genomic fingerprint of *Klebsiella* spp. using enterobacterial repetitive intergenic consensus (ERIC) polymerase chain reaction (PCR) by using ERIC1 (5-ATG TAA GCT CCT GGG GAT TCA C-3′) and ERIC2 (5′-AAG TAA GTG ACT GGG GTG AGC G-3′) primers [6]. The reaction mix contained 50 ng template of DNA, 1 × PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM, $MgCl_2$, 0.1% Triton X-100), 2.5 mM dNTP, 2.5 U Taq polymerase (Roche Diagnostics, Mannheim, Germany) and 5 mM of each primer (ERIC1 and ERIC2) in a final volume of 50 μl [7]. The amplification procedure consisted of the following cycling steps: initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 2 min. The final extension step was performed at 72°C for 2 min.

Gel electrophoresis and data analysis

We analyzed PCR products using 1.8% agarose gel electrophoresis (Scie-Plas; Warwickshire, UK) by using TBE 1× buffer (0.9 M Tris, 0.9 M Boric acid, and 20 mM EDTA, pH 8.3) at 90 V for 5 h. Agarose gels were documented using a Gel Logic 200 Molecular Imaging System (Kodak; Rochester, NY, USA). To analyze the profiles of *Klebsiella* strains, NTSYSpc (version 2.1; Applied Biostatistics, Port Jefferson, NY, USA) Numerical Taxonomy and Multivariate Analysis System was used and a dendrogram was constructed using an unweighted pair group method with arithmetic mean and by using Dice coefficients of similarity. *Klebsiella sp.* strains with a similarity coefficient of 70% were grouped in a genotype.

Ethical considerations

This study was designed to fully protect the anonymity of patients using unlinked anonymized samples and was conducted in compliance with the principles of the contemporary version of the Declaration of Helsinki. The protocol was approved by the Hacettepe University Scientific Research Projects Coordination Unit (project No. 08D11601001).

Results

We found that the urine samples and the urology service unit were the source of most *Klebsiella* spp. isolated with *K. pneumoniae*, *K. ornithinolytica*, and *K. terrigena* species being the most frequent (**Figures 1** and **2**).

The greatest resistance was observed against trimethoprim-sulfamethoxazole (88%), the least resistance was against imipenem (12%) as shown in **Table 1**.

Eleven different antibiotypes were generated. AI was the most frequently observed antibiotype, AVII and AVIII were only observed in *K. pneumoniae*, AIII and AVI were only observed in *K. ornithinolytica*, AX was only observed in *K. rhinoscleromatis*, and AIX was only observed in *K. oxytoca*. (**Figure 3**).

Accordingly, 25% of all *Klebsiella* spp. strains were found as MDR and 65% of these were isolated from urine. MDR strains were mostly isolated from the species of *K. ornithinolytica* (35%) followed by *K. pneumoniae* (29%) and they grouped in AV antibiotype profile (53%).

Genotyping performed by using ERIC/PCR grouped *Klebsiella* spp. strains into 23 different genotypes with a similarity coefficient of 70% (**Figure 4**). Only 43% of all genotypes included MDR strains. Accordingly, 50% of the strains in the 4th genotype (K31, K32, K36, K38) were found as MDR and all of the MDR strains of *K. terrigena* were grouped in the AV antibiotype profile. In the 9th genotype, there were only two MDR strains (K5, K10) and they belonged to *K. ornithinolytica* (**Figure 4**).

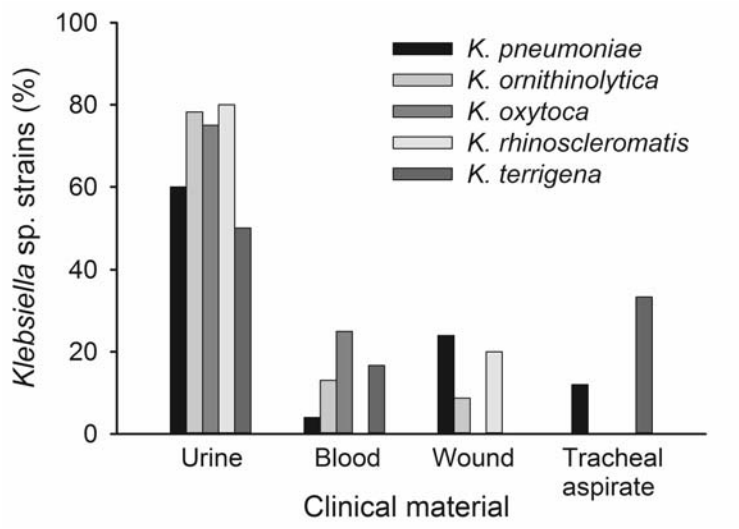


Figure 1. *Klebsiella* species found in various clinical materials.

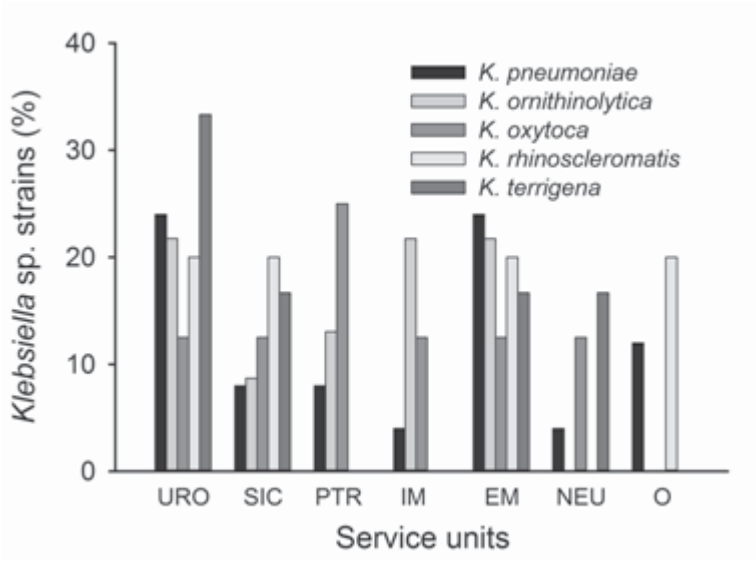


Figure 2. *Klebsiella* species found in various service units
URO, urology; SIC, surgical intensive care unit; PTR, physical treatment and rehabilitation unit; IM, internal medicine; EM, emergency medicine; NEU, neurology unit; O, otorhinolaryngology

Table 1. Antibiotic resistance of *Klebsiella* species

Antibiotics	***K. pneumoniae***	***K. ornithinolytica***	***K. oxytoca***	***K. rhinoscleromatis***	***K. terrigena***	**All *Klebsiella* spp. (%)**
Ciprofloxacin	16%	13%	13%	20%	0%	13%
Imipenem	12%	22%	0%	20%	0%	12%
Ampicillin-sulbactam	24%	35%	0%	20%	50%	28%
Piperacillin-tazobactam	20%	35%	38%	20%	50%	31%
Ceftizoxime	16%	13%	0%	20%	50%	18%
Trimethoprim-sulfamethoxazole	84%	87%	100%	20%	100%	88%

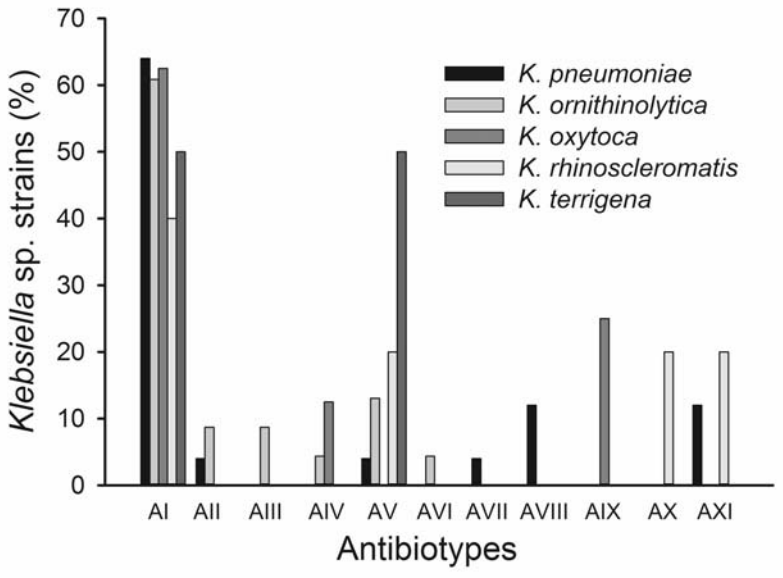


Figure 3. Antibiotype profiles of *Klebsiella* species
Antibiotypes belong to antibiotic resistance to: **AI** SXT; **AII** SAM, IPM, TZP; **AIII** SAM, IPM, TZP, CIP, SXT; **AIV** TZP, CIP, SXT; **AV** SAM, TZP, CZ, SXT; **AVI** SAM, IPM; **AVII** SAM, IPM, CIP, SXT; **AVIII** SAM, IPM, CIP, SXT, CZ; **AIX** SXT, TZP; **AX** SAM, CZ, CIP, SXT; **AXI** TZP. (SXT: trimethoprim-sulfamethoxazole (1.25/23.75 μg), SAM: ampicillin-sulbactam (10/10 μg), IPM: imipenem (10 μg), TZP: piperacillin tazobactam (100/10 g), CIP: ciprofloxacin (5 μg), CZ: ceftizoxime (30 μg))

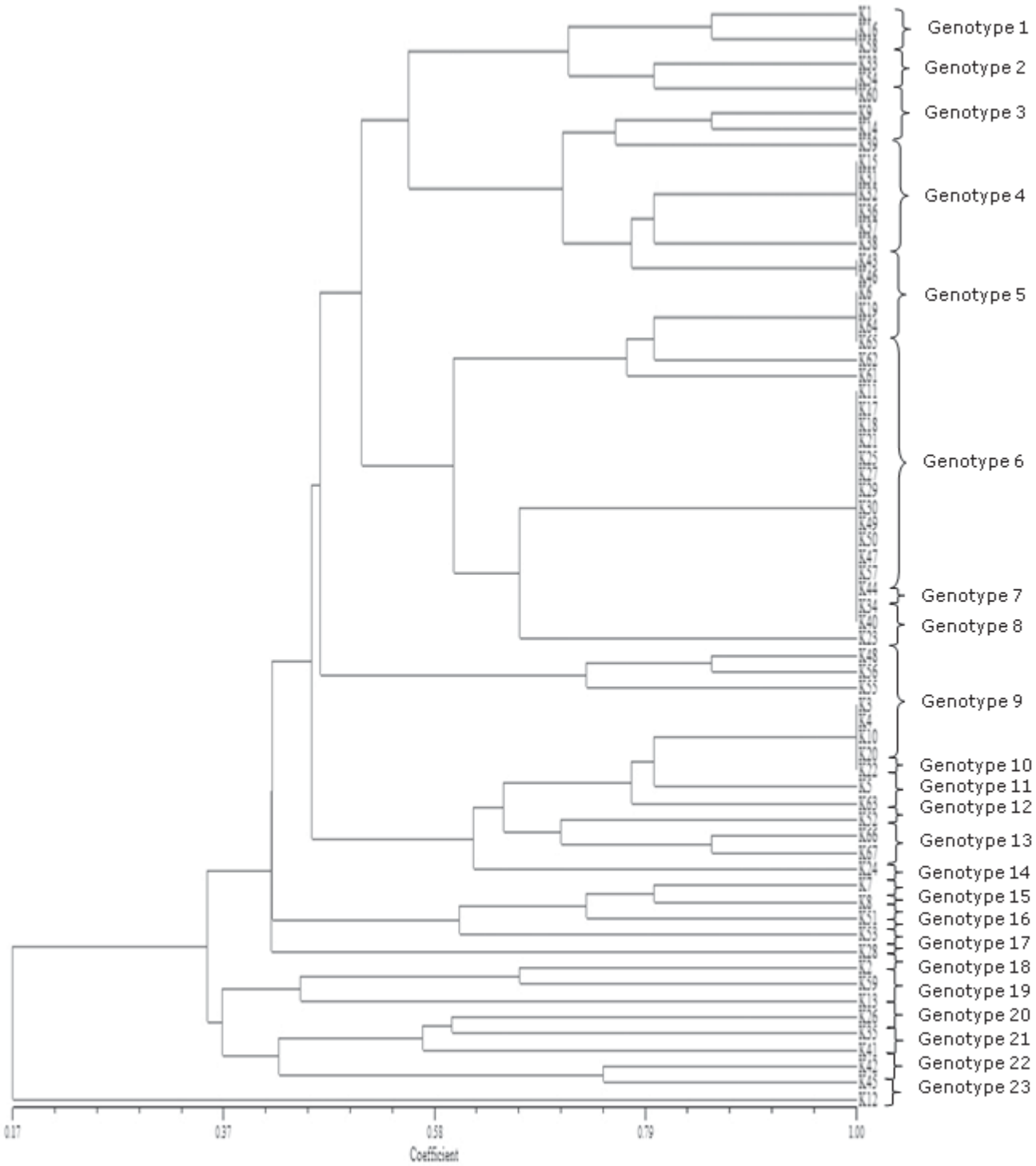
Discussion

Klebsiella spp. are known as an important opportunistic pathogens among the members of the *Enterobacteriaceae* [1, 8-11]. Accordingly, *Klebsiella* spp. are the second most causative agent of urinary tract infections (UTIs) after *Escherichia coli*. They represent 6%–17% of all nosocomial UTIs and 7% of all nosocomial infections [9, 12, 13]. Additionally, *Klebsiella* sp. are known as major agents complicating symptomatic or asymptomatic catheter-acquired UTIs; especially in hospitalized patients [14]. We identified the major foci where these infections occurred and were able to institute defensive procedures against additional spread. As consistent with previously published findings, we found that *Klebsiella* infections were widespread in samples of urine and in UTIs when compared with other clinical materials [9, 10, 15, 16]. We also found that the most effective antibiotic against *Klebsiella* spp. was imipenem. The least effective antibiotic was trimethoprim-sulfamethoxazole, and that the overall pattern of imipenem resistance was low: 0% [17], 2% [18], 5.4% [19], 13.9%, [10], and 16% [20]. Additionally, we found 25% of all *Klebsiella* sp. in the present study to be MDR and that strains that belong to *K. ornithinolytica* (35%) were predominantly MDR, followed by *K. pneumoniae* (29%). Previously, *K. pneumoniae* was found to be the predominant MDR species [21].

Various molecular typing methods, especially PCR fingerprinting assays, are now increasingly being used to identify strains in clinical practice [22]. ERIC/PCR showed that there was heterogeneity between genotypes of *Klebsiella* spp. strains and that there was no correlation between different species according to their ERIC/PCR profiles. Similarly, phylogenetic analysis of *Klebsiella* in a previous study showed taxonomic heterogeneity among the species [23]. Electrophoretic analysis of *K. pneumoniae* also showed genotypical heterogeneity [24-26]. It is therefore not surprising to find genotypical heterogeneity among our five different *Klebsiella* species, which were isolated from various clinical materials and service units, and grouped in 11 different antibiotypes.

Conclusions

Imipenem was the most effective antibiotic against *Klebsiella* spp. in our geographical region of Turkey. MDR strains are widespread among *Klebsiella* spp., especially among *K. ornithinolytica* and *K. pneumoniae*. Genotypical heterogeneity exists



K1–K15, K33–K38, K57, K58: K. ornithinolytica; K16–K21, K39–K49, K59–K66: *K. pneumoniae*; K22–K29: *K. oxytoca*; K30, K54–K56, K67: *K. rhinoscleromatis*; K31–K32, K50–K53: *K. terrigena*

Figure 4. Cluster analysis of the profiles obtained from five different *Klebsiella* species by ERIC-PCR analysis

among *Klebsiella* spp. It is important to be vigilant for nosocomial infections and to take precautions against the transmission of infectious microorganisms between hospitalized patients to prevent the emergence of MDR strains.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

References

1. Akter J, Chowdhury AMMA, Al Forkan M. Molecular study on plasmid profiling of clinical isolates of antibiotic resistant *Klebsiella* from Chittagong city. Int J Chem Life Sci. 2013; 2:1163-5.
2. Cartelle M, Tomas MM, Pertega S, Beceiro A, Dominguez MA, Velasco D, et al. Risk factors for colonization and infection in a hospital outbreak caused by a strain of *Klebsiella pneumoniae* with reduced susceptibility to expanded-spectrum cephalosporins. J Clin Microbiol. 2004; 4242-9.
3. Villanueva R, Bou G, Woods CR, Versalovic J, Koeuth T, Lupski JR. Whole-cell repetitive element sequence-based polymerase chain reaction allows rapid assessment of clonal relationships of bacterial isolates. J Clin Microbiol. 1993; 31:1927-31.
4. Avcioglu (*n e* Urkmez) NH. Investigation and typing of antibiotic resistance and plasmid profiles of *Klebsiella* strains. 94pp. MSc Dissertation. Ankara, Turkey: Graduate School of Science and Technology, Hacettepe University; 2009.
5. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2011; 1-14.
6. Duan H, Chai T, Liu J, Zhang X, Qi C, Gao J, et al. Source identification of airborne *Escherichia coli* of swine house surroundings using ERIC-PCR and REP-PCR. Environ Res. 2009; 109:511-7.
7. Idil N, Bilkay IS. Application of RAPD-PCR for determining the clonality of methicillin resistant *Staphylococcus aureus* isolated from different hospitals. Braz Arch Biol Tech. 2014; 57:548-53.
8. Bush K. Alarming β-lactamase-mediated resistance in multidrug-resistant *Enterobacteriaceae*. Curr Op Microbiol. 2010; 13:558-64.
9. Podschun R, Ullman U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 1998; 11: 589-603.
10. Ashour HM, El-Sharif A. Species distribution and antimicrobial susceptibility of Gram-negative aerobic bacteria in hospitalized cancer patients. J Trans Med. 2009; 19:7-14.
11. Gaynes R, Edwards JR, the National Nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis. 2005; 41:848-54.
12. Lina TT, Rahman SR, Gomes DJ. Multiple-antibiotic resistance mediated by plasmids and integrons in uropathogenic *Escherichia coli* and *Klebsiella pneumoniae*. Bangladesh J Microbiol. 2007; 24:19-23.
13. Hansen DS, Aucken HM, Abiola T, Podschun R. Recommended test panel for differentiation of *Klebsiella* species on the basis of a trilateral interlaboratory evaluation of 18 biochemical tests. J Clin Microbiol. 2004; 3665-9.
14. Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. Disease a Month. 2003; 49:71-82.
15. Raymond J, Aujard Y. Nosocomial infections in pediatric patients a European Multicenter Prospective Study. Infection Control. 2000; 21:260-3.
16. Kil KS, Darouiche RO, Hull RA, Mansouri MD, Musher DM. Identification of a *Klebsiella pneumoniae* strain associated with nosocomial urinary tract infection. J Clin Microbiol. 1997; 35:2370-4.
17. Mathaia D, Jonesa RN, Pfallera MA; SENTRY Participant Group North America. Epidemiology and frequency of resistance among pathogens causing urinary tract infections in 1,510 hospitalized patients: A report from the SENTRY Antimicrobial Surveillance Program (North America). Diag Microbiol Infect Dis. 2001; 40:129-36.
18. Snitkin ES, Zelazny AM, Thomas PJ, Stock F; NISC comparative sequencing program, Henderson DK, et al. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. Sci Transl Med. 2012; 22:4.
19. Saied T, Elkholy A, Hafez SF, Basim H, Wasfy MO, El-Shoubary W, et al. Antimicrobial resistance in pathogens causing nosocomial bloodstream infections in university hospitals in Egypt. Am J Infect Control.

2011; 39:61-5.
20. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, et al. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. Arch Intern Med. 2005; 165:1430-35.
21. Hasdemir UO, Chevalier J, Nordmann P, Pages JM. Detection and prevalence of active drug efflux mechanism in various multidrug-resistant *Klebsiella pneumoniae* strains from Turkey. J Clin Microbiol. 2004; 42:2701-6.
22. Giesendorf BA, Belkum A, Koeken A, Stegeman H, Henkens MH, van der Plas J, et al. Development of species-specific DNA probes for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* by polymerase chain reaction fingerprinting. J Clin Microbiol.1993; 31:1541-6.
23. Granier SA, Leflon-Guibout V, Goldstein FW, Nicolas-Chanoine M-H. Enterobacterial repetitive intergenic consensus 1R PCR assay for detection of *Raoultella* sp. isolates among strains identified as *Klebsiella oxytoca* in the clinical laboratory. J Clin Microbiol. 2003; 41:1740-2.
24. Lim KT, Yeo CC, Yasin RM, Balan G, Thong KL. Characterization of multidrug-resistant and extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strains from Malaysian hospitals. J Med Microbiol. 2009; 58:1463-9.
25. Ben-Hamouda T, Foulon T, Ben-Cheikh-Masmoudi A, Fendri C, Belhadj O, Ben-Mahrez K. Molecular epidemiology of an outbreak of multiresistant *Klebsiella pneumoniae* in a Tunisian neonatal ward. J Med Microbiol. 2003; 52:427-33.
26. Pai H, Kang CI, Byeon JH, Lee KD, Park WB, Kim HB, et al. Epidemiology and clinical features of bloodstream infections caused by AmpC-type-β-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004; 48:3720-8.