

# Antibiotic resistance and genotyping of gram-negative bacteria causing hospital-acquired infection in patients referred to Children's Medical Center

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Setareh Mamishi<sup>1,2</sup>  
Shima Mahmoudi<sup>2</sup>  
Naimeh Naserzadeh<sup>1</sup>  
Reihaneh Hosseinpour Sadeghi<sup>2</sup>  
Mohammad Taghi Haghi  
Ashtiani<sup>3</sup>  
Abbas Bahador<sup>4</sup>  
Mohammad Reza Abdosalehi<sup>1</sup>  
Maryam Rahmani<sup>5</sup>  
Babak Pourakbari<sup>2</sup>

<sup>1</sup>Department of Infectious Diseases, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; <sup>2</sup>Pediatric Infectious Disease Research Center, Tehran University of Medical Science, Tehran, Iran;

<sup>3</sup>Department of Pathology, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; <sup>4</sup>Department of Microbiology, Tehran University of Medical Sciences, Tehran, Iran; <sup>5</sup>Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

**Background and aim:** Hospital-acquired infection (HAI) is a major problem worldwide. Understanding patterns of bacterial etiology and antibiotic susceptibility is vital to combating HAI. Besides, typing of isolates is important to establish the intra-hospital surveillance of resistant clones. In this study, we aimed to evaluate antibiotic resistance and genotyping of a number of gram-negative bacteria (GNB) causing HAI in patients who were referred to Children's Medical Center.

**Methods:** During the 6-month period, antimicrobial susceptibility profiles of GNB isolates recovered from patients in Children's Medical Center were determined. Typing of common GNB was performed by random amplified polymorphic DNA (RAPD) analysis and the results were analyzed by Gelcompar II software.

**Results:** In total, 142 (1.1%) gram-negative bacterial strains were isolated, among which 59.2% were from males. The most organisms were isolated from blood (63%) followed by wounds (13.7%). The greatest proportion of strains came from intensive care units (51%). Low sensitivity of *Acinetobacter baumannii* to common antibiotics and high resistance of *Enterobacter* spp. To cefotaxime (100%) were reported. The most efficient antibiotics for *Escherichia coli* strains were amikacin (84%) and gentamycin (81%). However, just 12.5% of *Serratia marcescens* strains were resistant to trimethoprim-sulfamethoxazole. The analysis of RAPD-typing revealed the presence of one clone in *A. baumannii* and *E. coli* and two clones in *Klebsiella pneumoniae*, whereas the trend varied completely in *Pseudomonas aeruginosa* strains and *Enterobacter* spp.

**Conclusion:** The results of this study showed a possibility of an outbreak in the Children's Medical Center. Since there is a possibility of transmission of an infection from one patient to another, high attention should be paid to the basic methods of preventing infection.

**Keywords:** hospital-acquired infection, gram-negative bacteria, antibiotic resistance

## Introduction

Antimicrobial resistance among gram-negative bacteria (GNB) is growing worldwide.<sup>1</sup> It is a main public health problem causing both significant morbidity and mortality among hospitalized patients. A direct correlation between resistance of GNB and patient mortality, cost of patient care, and length of hospital stay has been shown.<sup>2,3</sup> Lots of gram-negative organisms are responsible for hospital-acquired infections (HAIs), among which the *Enterobacteriaceae* family is the most commonly recognized group.

Multidrug-resistant organisms such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and ESBL-producing or carbapenemase-producing *Enterobacteriaceae*, are increasingly being reported worldwide.<sup>4</sup>

Correspondence: Babak Pourakbari  
Pediatric Infectious Disease Research  
Center, Children Medical Center  
Hospital, Tehran University of Medical  
Sciences, 62 Gharib Street, Keshavarz  
Blvd, Tehran, Iran  
Tel +98 21 6642 8996  
Fax +98 21 6642 8996  
Email pourakbari@tums.ac.ir

It is essential to understand pathogen distribution and relatedness in order to determine the epidemiology of HAIs.<sup>5</sup> There have been various technical approaches to bacterial source tracking,<sup>6,7</sup> but a consensual single method for field application has not been recognized until now.<sup>8</sup> Genomic fingerprinting methods are now regarded as the most accurate methods for the typing of microorganisms.<sup>9</sup> These methods comprise pulsed field gel electrophoresis, ribotyping, and PCR-based fingerprinting methods.<sup>10</sup> The aim of our study was to evaluate antibiotic resistance of GNB and genotyping of common GNB causing HAI in patients referred to Children's Medical Center during 6 months by random amplified polymorphic DNA (RAPD) PCR.

## Material and methods

The current study was carried out in the referral hospital of Children's Medical Center, Tehran, Iran between July 2017 and January 2018. The whole GNB strains isolated from clinical specimens of blood, wounds, and sterile fluids of patients hospitalized in this center were used in study. Ethical approval was obtained from the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran. GNB isolates were not specifically isolated for this research and were part of the routine hospital laboratory procedure. The BACTEC 9120 Blood Culture System (BD, Franklin Lakes, NJ, USA) was used for quick detection of microorganisms in blood samples. Gram staining and subculture of the samples were performed on MacConkey agar, chocolate agar, and blood agar plates. Identification of microorganisms was carried out using conventional biochemical methods. The tests used were a Kligler iron agar slant, catalase and oxidase tests, sugar fermentation, Simmons' citrate agar slant, urea hydrolysis slant, methyl red/Voges-Proskauer test, and motility test.

Antimicrobial resistance of each organism was assessed using standard techniques according to Clinical and Laboratory Standards Institute criteria.<sup>11</sup> The evaluated antibiotics include piperacillin-tazobactam, imipenem, meropenem, gentamicin, amikacin, cefepime, ceftazidime, colistin, ciprofloxacin, cefotaxime, and trimethoprim-sulfamethoxazole.

In order to accomplish the genotyping of the common hospital-acquired GNB (including *A. baumannii*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Escherichia coli*, and *Enterobacter* spp.), bacterial genomic DNA extraction was performed using DNA extraction kit (Bioneer, Daejeon, South Korea) according to manufacturer's guidelines. RAPD-PCR was done as described previously.<sup>5</sup> Briefly, DNA amplification was accomplished on a thermo cycler

(Bio-Rad Laboratories Inc., Hercules, CA, USA) in a final volume of 25 mL containing 1× PCR buffer, 50 mM MgCl<sub>2</sub>, 10 mM dNTP mix, 50 mM primer 272 (3'-AGCGGGCCAA-5'), 12.1 unit Taq polymerase (Qiagen NV, Venlo, the Netherlands), doubledistilled water, and genomic DNA equivalent to 40 ng.<sup>5</sup>

The RAPD-PCR was carried out under the following conditions: initial four cycles of denaturation at 94°C for 5 minutes, annealing at 36°C for 5 minutes, extension at 72°C for 5 minutes followed by 30 more cycles of denaturation at 94°C for 1 minute, annealing at 36°C for 1 minute, and extension at 72°C for 1 minute. The RAPD-PCR products were loaded on a 1% (w/vol) agarose gel containing 1/10,000 gel red (Biotium, Fremont, CA, USA), and were analyzed by gel electrophoresis and banding patterns were visualized and photographed in Geldocumentation system (Uvitec, Cambridge, UK). A 100 bp DNA ladder (Thermo Fisher Scientific, Waltham, MA, USA) was used as a molecular size standard. SPSS software (version 13; SPSS Inc., Chicago, IL, USA) was used for data analysis and RAPD-PCR fingerprinting profile was analyzed with Gelcompar II software, version 6.5 (Applied Maths, Sint-Martens-Latem, Belgium). Similarity analysis of results was calculated using the Dice coefficient/unweighted pair-group method with arithmetic mean. The criteria for related clones were taken as profiles with 80% or more similar bands. Isolates with 100% similarity were considered as the same RAPD-PCR type.

## Results

In the current study, 142 (2.2%) GNB strains were isolated from 6,524 blood, wound, and sterile body fluid cultures. The GNB were isolated from 84 boys (59.2%) and 58 girls (40.8). The median age of the patients was 11.4 months (IQR: 1.9 months-4 years) (Table 1). Ninety-one patients (64%) had a history of previous hospitalization and 13 patients (9%) had died by the end of the study.

As shown in Table 1, the most isolates were collected from surgical unit and intensive care units (ICUs) (particularly EICU and Neonatal Intensive Care Unit). Forty-four patients (31%) utilized ventilators, 80 patients (56%) used catheters, and 12 of them (8.5%) had positive central venous catheter tip culture during their hospitalization.

The most common isolated GNB was *E. coli* (n=32, 22/5%), followed by *K. pneumoniae* (n=29, 20%) and *Pseudomonas* spp. (n=17, 12%) (Table 2). A total of 142 organisms were isolated from blood (63%), wounds (13.7%), ascites (8.7%), cerebrospinal fluid (6%), central venous catheter tip (5%), dialysis fluid (1%), pleural fluid (0.7%), and the

**Table I** Characteristics of studied patients

Characteristics		N (%)
Gender	Male	84 (59.2)
	Female	58 (40.8)
Age (years)	<1	71 (50)
	1–5	45 (31.7)
	>5	26 (18.3)
Ward	Surgical	19 (13)
	Emergency	17 (12)
	EPICU	14 (10)
	NICU	13 (9)
	PICU	12 (8.5)
	NICU OPH	10 (7)
	Nephrology	8 (6)
	Oncology	8 (6)
	Infectious	7 (5)
	Gastroenterology	7 (5)
	CICU	7 (5)
	Rheumatology	5 (3.5)
	Neonatal	4 (3)
	IICU	3 (2)
	Urology	3 (2)
	Neurology	2 (1)
	Cardiology	2 (1)
	Transplant	1 (0.7)

**Abbreviations:** EPICU, Emergency Intensive Care Unit; NICU, Neonatal Intensive Care Unit; PICU, Pediatric Intensive Care Unit; OPH, Ophthalmology; CICU, Cardiac Intensive Care Unit; IICU, Intermediate Intensive Care Unit.

other sterile cultures (1.4%). Fourteen strains were separated from peritoneal fluid in which *E. coli* with a number of nine strains (64%) was regarded as the most common.

Among all the gram-negative organisms, 100 isolates met the criteria of nosocomial infection. All of the *S. marcescens*, *A. baumannii*, *Enterobacter* spp., and *P. aeruginosa* isolates met the criteria of HAI.

Forty-one strains were from ICUs, most of which were *S. marcescens* (N=13, 22%), *K. pneumoniae* (N=12, 21.3%), *E. coli* (N=9, 15.2%), and *A. baumannii* (N=8, 13.5%). While the most prevalent isolates in the surgical unit were *E. coli* (N=11, 57.9%). HAI was reported in all the patients with positive

culture of central venous catheter tip, 79 patients (99%) with catheters, and 43 patients (98%) who had used a ventilator. Interestingly, 99% of children who met the criteria of HAI had a history of previous hospitalization.

## Antibiotic resistance

Antibiotic resistance frequencies of evaluated GNB were depicted in Table 3. High frequency of antibiotic resistance to cefotaxime (81%) and trimethoprim-sulfamethoxazole (75%) was seen in *E. coli* strains, whereas the most sensitive antibiotics were amikacin (84%) and gentamycin (81%). *K. pneumoniae* strains were highly resistant to cefotaxime (96.5%) and cefepime (86%) and sensitive to imipenem (65%), amikacin (64%), and gentamycin (57%). Likewise, in *Klebsiella oxytoca*, high resistance to the same antibiotics (75%) was seen. High sensitivity to gentamycin and trimethoprim-sulfamethoxazole was reported (75%) and all of them were sensitive to imipenem. Besides, the low sensitivity of *A. baumannii* to common antibiotics and high resistance of *Enterobacter* spp. to cefotaxime (100%) was reported (Table 3).

## Genotyping

The genotyping of *A. baumannii* causing HAI showed the presence of one clone in which two clusters with high similarity (95%) were present (Figure 1A). Among ten *A. baumannii* strains, five strains had 100% similarity and it might be due to the development of one strain in the Children's Medical Center. The genotyping of *E. coli* and *K. pneumoniae* (Figure 1B and C) demonstrated the presence of one and two clones, respectively with  $\geq 80\%$  genetic affinity. However, the trend varied completely in *P. aeruginosa* strains and *Enterobacter* spp. (Figure 1D and E). Among 15 isolates of *P. aeruginosa*, just two isolates had 100% genetic similarity and four strains showed  $\geq 80\%$  similarity.

## Discussion

We assessed the antimicrobial resistance of GNB recovered from blood, wounds, and sterile fluids from patients who were referred to the Children's Medical Center and the genotyping of the isolated strains from the children who had HAI.

In the current study, the most frequent GNB were *E. coli* (n=32, 22/5%), *K. pneumoniae* (n=29, 20%), *Pseudomonas* spp. (n=17, 12%), *S. marcescens* (n=15, 11%), *S. maltophilia* (n=11, 8%), *A. baumannii* (n=10, 7%), and *Enterobacter* spp. (n=6, 4%). This report is similar to our previous study<sup>13</sup> with the exception of the higher prevalence of *K. pneumoniae* compared to *E. coli*. In another study performed by

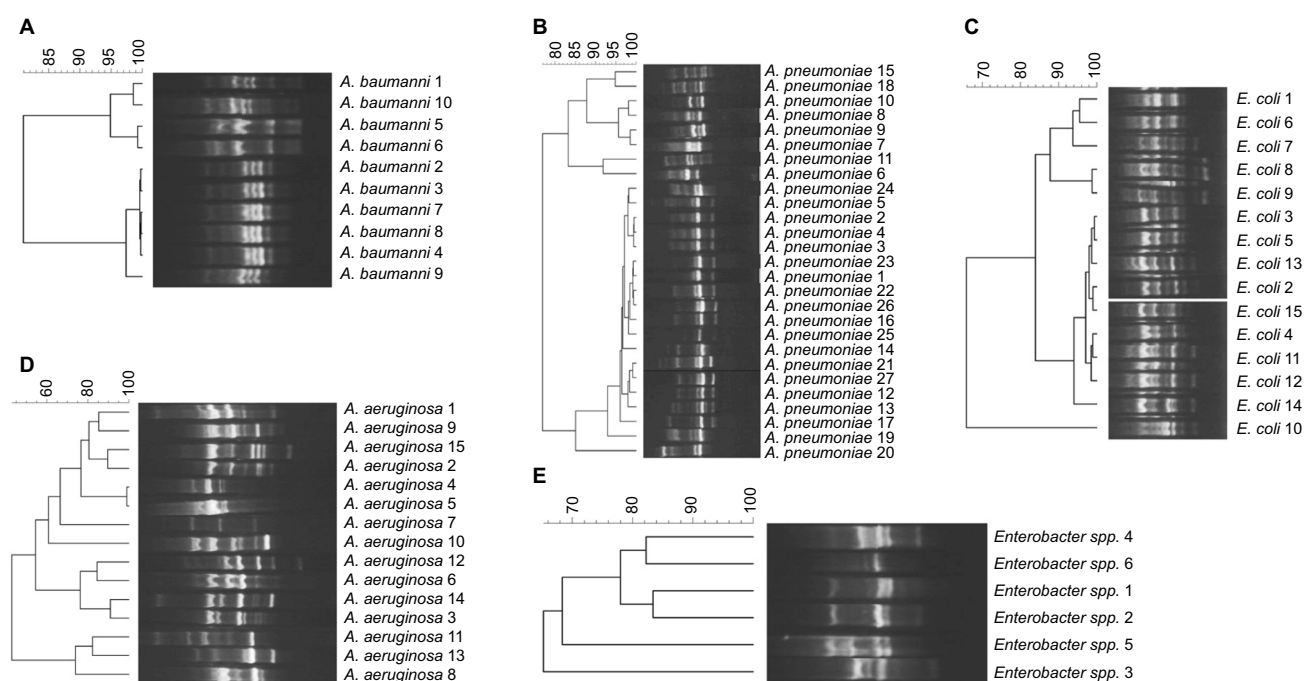
**Table 2** Frequency of occurrence of GNB isolated from blood, wounds, and sterile body fluid cultures

Sample	<i>Escherichia coli</i> N (%)	<i>Klebsiella pneumoniae</i> N (%)	<i>Klebsiella oxytoca</i> N (%)	<i>Pseudomonas</i> spp. N (%)	<i>Serratia marcescens</i> N (%)	<i>Stenotrophomonas maltophilia</i> N (%)	<i>Acinetobacter baumannii</i> N (%)	<i>Enterobacter</i> spp. N (%)	<i>Pseudomonas aeruginosa</i> N (%)	<i>Morganella morganii</i> N (%)	<i>Salmonella</i> spp. N (%)	Total N (%)
Blood	15 (17)	19 (21)	2 (2)	15 (17)	10 (11)	11 (12)	6 (7)	4 (4)	6 (7)	1 (1)	1 (1)	90 (63)
Wound	6 (30)	2 (10.5)	1 (5)	0 (0)	2 (10.5)	0 (0)	2 (10.5)	0 (0)	7 (37)	0 (0)	0 (0)	20 (13.7)
Abdominal fluid	8 (89)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)	9 (6.7)
CSF	2 (25)	2 (25)	0 (0)	2 (25)	0 (0)	0 (0)	0 (0)	1 (12.5)	1 (12.5)	0 (0)	0 (0)	8 (6)
Central venous catheter tip	0 (0)	2 (28.5)	0 (0)	0 (0)	2 (28.5)	0 (0)	2 (28.5)	1 (14)	0 (0)	0 (0)	0 (0)	7 (5)
Ascitic fluid	1 (33)	1 (33)	1 (33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (2)
Dialysis fluid	0 (0)	1 (50)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)
Other sterile fluids	0 (0)	(100)2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.4)
Pleural fluid	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)
Total	32 (22.5)	29 (20)	4 (3)	17 (12)	16 (11)	11 (8)	10 (7)	6 (4)	15 (11)	1 (0.7)	1 (0.7)	142 (100)

**Abbreviations:** GNB, gram-negative bacteria; CSF, cerebrospinal fluid.

**Table 3** In vitro antimicrobial resistance of evaluated gram-negative bacteria

Bacteria	Piperacillin-Tazobactam	Imipenem	Meropenem	Gentamicin	Amikacin	Cefepime	Cefotaxime	Trimethoprim-Sulfamethoxazole	Colistin	Ceftazidime	Ciprofloxacin	Ampicillin-sulbactam
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
<i>Escherichia coli</i>	13/31 (42)	2/6 (33)	2/3 (67)	6/31 (19)	5/31 (16)	21/32 (66)	26/32 (81)	24/32 (75)	–	–	–	–
<i>Klebsiella pneumoniae</i>	16/26 (61.5)	6/17 (35)	1/1 (100)	15/28 (53.5)	13/28 (46)	25/29 (86)	28/29 (96.5)	19/29 (65.5)	0/6 (0)	–	–	–
<i>Serratia marcescens</i>	13/16 (81)	1/14 (7)	–	13/13 (100)	13/16 (81)	15/16 (94)	16/16 (100)	2/16 (12.5)	0/1 (0)	–	–	–
<i>Pseudomonas</i> spp.	1/15 (7)	2/15 (13)	–	13/15 (87)	13/15 (87)	10/15 (67)	4/15 (27)	–	–	–	–	–
<i>Klebsiella oxytoca</i>	1/4 (25)	0/1 (0)	–	1/4 (25)	3/4 (75)	3/4 (75)	3/4 (75)	1/4 (25)	–	–	–	–
<i>Acinetobacter baumannii</i>	8/10 (80)	8/10 (80)	–	8/10 (80)	8/10 (80)	8/10 (80)	10/10 (100)	8/10 (80)	0/8 (0)	8/10 (80)	7/8 (87.5)	6/8 (75)
<i>Enterobacter</i> spp.	3/6 (50)	–	–	1/6 (17)	0/6 (0)	3/6 (50)	4/4 (100)	1/4 (25)	–	–	–	–
<i>Pseudomonas aeruginosa</i>	0/15 (0)	3/15 (20)	1/1 (100)	1/15 (7)	0/15 (0)	0/15 (0)	–	–	–	1/15 (0)	–	–



**Figure 1** Genotyping of isolated *Acinetobacter baumannii* (A), *Klebsiella pneumoniae* (B), *Escherichia coli* (C), *Pseudomonas aeruginosa* (D), and *Enterobacter* spp. (E) Strains that met hospital-acquired infection criteria.

Mahmoudi et al,<sup>14</sup> *K. pneumoniae* (n=263, 27.5%) was reported as the most frequent GNB.

The most isolated bacteria were collected from blood-stream (63%), followed by wounds (13%). Primary blood-stream infections (28%), pneumonia (21%), and urinary tract infections (15%) were found to be most frequent in Richards et al's study.<sup>15</sup> It is obvious from the reported data that all the children with a history of hospitalization and almost all of the patients who used an invasive device met the criteria of nosocomial infection. *K. pneumoniae* was the most common organism isolated from blood culture (21%). However, this organism turned out to be *Enterobacter* spp. in Richards et al's study<sup>15</sup> and *P. Aeruginosa* in Murni et al's study.<sup>16</sup>

The greatest proportion of isolated strains was from ICUs (particularly EICU and Neonatal Intensive Care Unit) (51%). Likewise, in Folgori et al's study, the most GNB in Europe were isolated from Pediatric Intensive Care Unit/Cardiac Intensive Care Unit (22%).<sup>17</sup>

*A. baumannii* strains were significantly resistant to almost all tested antibiotics, which is similar to previous studies,<sup>14,18</sup> but different from Jafari et al's study<sup>19</sup> in which resistance rate to imipenem (41%), ciprofloxacin (78%), and meropenem (60%) was lower than ours. Whereas, in Ballot et al's study,<sup>20</sup> resistance rates to ceftazidime, ciprofloxacin, and gentamycin (15%, 11%, and 4%) were lower than we reported. *A.*

*baumannii* strains were sensitive to colistin and ampicillin-sulbactam. The resistance rate of *P. aeruginosa* in the current study, compared to the previous reports in the Children's Medical Center,<sup>13,14,21</sup> was substantially lower and showed quite high sensitivity to the tested common antibiotics. The *K. pneumoniae* strains depicted high resistance rate to cefotaxime (96.5%), which was compatible with the results reported by Molana et al (90%).<sup>22</sup> Various studies show that many resistant strains of bacteria that cause nosocomial infections are caused by an initial strain and expanded subsequently in the hospital environment between patients, or even transmitted by patients from different hospitals to each other.

Typing of bacteria is vital for monitoring newly emerging pathogens and for examining local outbreaks.<sup>23</sup> In this report, we used RAPD-PCR followed by agarose gel electrophoresis techniques for the molecular typing of common GNB collected during 6 months from the patients in the Children's Medical Center who had HAI. The standardization of the reaction conditions has contributed to making RAPD typing a trustworthy and reproducible technique,<sup>24</sup> with even more discriminatory power than DNA macro-restriction analysis by PFGE for bacterial typing.<sup>25</sup> However, random amplification could simply be applied for the surveillance and prevention of nosocomial infections by clinical microbiology laboratories by enhancing the resolution of the electrophoretic separation and the sensitivity of the staining.<sup>25</sup> The dendrogram of

genotyping of *A. baumannii* strains showed the presence of two clones with  $\geq 80\%$  genetic similarity. Besides, the low genetic similarity in *Enterobacter* spp. and *P. aeruginosa* could be plausible based on the high records of previous hospitalization of the patients. In previous studies carried out in the same center, the possibility of incidence of nosocomial infection was reported in presence of one bacterial clone.<sup>18,26</sup>

In previous studies which were conducted in our hospital, the analysis of genotyping of *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* strains showed the presence of one clone in the Children's Medical Center. Ninety-three percent of isolated *Klebsiella* spp. and all the *A. baumannii* strains that met the criteria of nosocomial infection were determined in one and three clusters, respectively with the high genetic similarity of  $\geq 80\%$ . In addition, 90% of *P. aeruginosa* strains were identified in one clone.<sup>18,26</sup>

In Wasfi et al's study,<sup>27</sup> RAPD-PCR showed 18 distinct patterns of *K. pneumonia* isolates with  $\geq 80\%$  similarity. In addition, the genotyping of *E. coli*, *K. pneumonia*, and *P. aeruginosa* demonstrated seven RAPD patterns for *E. coli*, five for each of *K. pneumonia* and *P. aeruginosa* isolates.<sup>28</sup> Likewise, according to Sachse et al's study<sup>29</sup> in 2014, RAPD is probably functional as a rapid screening method for the intra-hospital surveillance of *K. pneumoniae*, allowing discrimination of clonally related strains. In another study, Asadollahi et al<sup>30</sup> reported three predominant genotypes and ten unrelated genotypes of *A. baumannii*.

## Conclusion

The results of this study showed the presence of clones with  $\geq 80\%$  similarity in three common GNB, *E. coli*, *A. baumannii*, and *K. pneumoniae*, indicating occurrence of a possible outbreak in the Children's Medical Center. Since there is a possibility of transmission of an infection from one patient to another, much attention should be paid to the basic methods of preventing infection (standard precautions), disinfection of medical equipment, and personnel hygiene. The length of hospitalization and the use of antibiotics in patients, especially during long-term hospitalization, could have impacts on the reduction of antibiotic resistance and the occurrence of HAI.

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## Disclosure

The authors report no conflicts of interest in this work.

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