ORIGINAL RESEARCH

Evaluating the antimicrobial resistance patterns among major bacterial pathogens isolated from clinical specimens taken from patients in Mofid Children's Hospital, Tehran, Iran: 2013–2018

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Background: This study evaluates the epidemiology and antimicrobial resistance profile of Gram-negative bacteria (GNB) and Gram-positive bacteria (GPB) isolated from clinical specimens in children admitted to Mofid Children's Hospital.
Methods: This was a retrospective study of the patients' clinical specimens collected from January 2013 until the end of December 2018. All specimens were evaluated to determine the presence of infection-causing agents using a BACTEC 9120 blood culture. Isolation and identification of bacterial strains were performed using conventional biochemical tests. Antibiotic resistance was determined using Kirby–Bauer disk diffusion and broth microdilution methods. Results were interpreted according to CLSI and EUCAST.
Results: A total of 1130 different pathogenic bacteria were detected from 14,690 different clinical specimens and the overall detection rate was 7.7% (1130/14,690). Among bacterial pathogen isolated from clinical specimens, 55% (n=622) were GNB and 45% (n=508) were GPB. The predominant GNB isolates were *Pseudomonas aeruginosa, Klebsiella* spp., *Acinetobacter baumannii, Escherichia coli, Enterobacter* spp., *Citrobacter* spp., respectively. Among GPB, CoNS was the most frequent and *Enterococcus* spp. was found to have low levels of resistance to linezolid. In GNB, most *A. baumannii* and *P. aeruginosa* were ceftriaxone resistant. *P. aeruginosa* was found to have low levels of resistance to levofloxacin and ciprofloxacin.
Conclusions: Our findings revealed that the resistance rate among GNB and GPB associated with different infections in children is very high. These results suggest a constant screening and follow-up programs for the detection of antibiotic resistance, and it also suggests to develop antimicrobial stewardship programs in Tehran, Iran.
Keywords: Gram-negative bacteria, Gram-positive , bacteria, bacterial, infection, antimicrobial, resistance, children, Iran

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Introduction

Nowadays, antimicrobial resistance (AMR) is considered as a main public health threat,[1–5] also AMR bacteria in different hospital wards are increasing significantly.[6–8] Based on a published study, 700,000 deaths are reported annually due to AMR, and it has been predicted that if appropriate control and prevention measures are not taken, AMR would become one of the main reasons of death among hospitalized or non-hospitalized patients in developing and developed countries.[9] Proper antibiotic usage and administration are essential for treatment of bacterial infections.[10,11] Thus, inappropriate prescription and

misuse of antibiotics could contribute to the emergence of AMR pathogenic bacteria, restriction of therapeutic options, increase of hospitalization time and high treatment costs and finally a greater death rate.[12–14] Bacteria are one of the main causes of infections in humans.[15–17] Children are usually considered as the most vulnerable group to bacterial infections.[12,18] According to the global action plan on AMR endorsed by WHO, it is important to raise awareness on AMR through monitoring and research programs in different parts of the world.[19–21] AMR monitoring is critical and has several benefits including: 1) providing data on bacterial resistance rate, 2) helping select appropriate antibiotics and subsequently reduce AMR rate,[22] 3) reduction in hospitalization rate and treatment costs, and 4) decrease in death rate.[1,11] Therefore, the current study evaluates the epidemiology and AMR profile of the main pathogenic bacteria isolated from hospitalized children from January 2013 until the end of December 2018 in Mofid Children's Hospital, Tehran Iran.

Materials and methods
Study design and identification of microorganisms

This was a retrospective study of the patients' clinical specimens collected over a 6-year period from January 2013 until the end of December 2018 in Mofid children's hospital. This research used microbiological lab data of 14,690 various clinical specimens collected from different hospital wards in Mofid children's hospital in Tehran. Clinical specimens were collected in BACTEC standard culture vials and were incubated at 37° C in BACTEC automated system. Moreover, BACTEC system database was studied and after patients with positive samples were detected, some information related to these patients suchas sex, age, type of specimens and their hospitalized ward was obtained. In the next step, the positive samples were subcultured on specific medium including MacConkey agar, chocolate agar, mannitol salt agar and blood agar plates, and then Gram staining of bacterial colonies were performed. Isolation and identification of different bacterial strains of positive cultures were performed using conventional biochemical tests including IMVIC (Indole, Methyl red, Voges proskauer and Citrate) test, catalase and oxidase test, growth on Triple Sugar Iron Agar and Kligler Iron Agar, Bile esculin agar, SH2 production, motility test, growth on 6% NaCl and DNase test.

Antibiotic susceptibility testing

The antibiotic resistance of the isolates was determined using Kirby–Bauer disk diffusion method (DDM) and broth microdilution method (for evaluating colistin and PB susceptibility in Gram-negative bacteria (GNB)); the results of DDM method were then interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria.

Moreover, interpretation of colistin MIC results was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (susceptible, ≤2 mg/L; resistant, >2 mg/L). On the other hand, considering that CLSI and EUCAST do not have interpretive criteria for polymyxin B for *Enterobacteriaceae*, a breakpoint of >2 mg/L for resistant isolates and ≤2 mg/L for susceptible isolates were used. The antibiotic discs and powders were purchased from MAST Company and Sigma (Sigma–Aldrich, cat No. PZ0021). The Gram-positive and -negative bacterial isolates including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* subsp. *aureus* ATCC 25923 were used as quality control for DDM and MIC tests. Due to annual changes in hospital policies, the applied antibiotics for treatment of various bacterial infections varied too.

The antimicrobial susceptibility for GNB and Gram-positive bacteria (GPB) was determined using the following antibiotic disks: ciprofloxacin (CIP); piperacillin/tazobactam (PTZ); tetracycline (TET); chloramphenicol (CHL); amikacin (AK); cefotaxime (CTX); ceftazidime (CAZ); cefepime (CPM); levofloxacin (LEV); trimethoprim-sulfamethoxazole (SXT); tobramycin (TOB); doxycycline (DOX); meropenem (MRP); doripenem (DOR); imipenem (IPM); ampicillin-sulbactam (SAM); ceftriaxone (CRO); cefuroxime (CXM); ampicillin (AMP); amoxicillin-clavulanic acid (AUG); nitrofurantoin (NI); gentamicin (GM); nalidixic acid (NA); cefazolin (CZ); azithromycin (AZM); cefoxitin (FOX); oxacillin (OX); erythromycin (ERY); quinupristin-dalfopristin (RP); vancomycin (VA); cefdinir (CD); clindamycin (CLI); ofloxacin (OFX); linezolid (LZD); penicillin (PEN); clarithromycin (CLa); cefpodoxime (CPd); norfloxacin (NOR); ticarcillin-clavulanic acid (TIM); aztreonam (ATM). The results of the research were documented as either susceptible (S), intermediate (I) or resistant (R). According to the European Centre for Disease Prevention and Control (ECDC) and the US Centers for Disease Control and Prevention (CDC), the identification of multidrug-resistant (MDR) isolates was conducted and GNB were selected as MDR, which were resistant to at least one antimicrobial among at least three or more drug categories.

Statistical analysis

The patients' information such as gender, age, type of specimens, the hospitalized ward and antibiotic susceptibility profiles were all collected from the hospital database and were analyzed using the statistical package SPSS v.23.0 (SPSS Inc., Chicago, IL, USA).

Results

Number and distribution of specimens and positive cultures

During this 6-year period, a total of 14,690 different clinical cultures were collected from January 2013 until the end of December 2018. Among which, 1130 (7.7%) cultures were positive from which various bacteria were isolated. Among GPB, about 58.8% and 41.2% of the total positive cultures were from male and female samples, respectively. On the other hand, among GNB, approximately 55% and 45% of the total positive cultures belonged to male and female samples, respectively (Table 2). In positive cases, the mean age was 11.2 years old (1 month to 15 years old). Various bacteria recovered from different hospital wards are: Hematology (n=151; 13.3%), Emergency (n=43; 3.8%), Gastroenterology (n=139; 12.3%), ICU (n=22; 2%), Infectious (n=287; 25.3%), Nephrology (n=33; 3%), Neurology (n=35; 3%), NICU (n=36; 3.1%), Renal unit (n=14; 1.2%), PICU (n=242; 21.4%), Rheumatology (n=15; 1.3%), Surgery (n=97; 8.5%), Oncology (n=10; 0.8%) and Urology (n=2; 0.17%). The frequency of various clinical samples isolated from bacterial strains was as follows: blood (n=1003; 88.7%), cerebrospinal fluid (CSF) (n=61; 5.3%), pleural fluid (n=46; 4%), dialysis fluid (n=1; 0.09%), luminal sample (n=8; 0.7%) and shunt (n=11; 1%). The frequency of GNB and GPB in various positive clinical samples and different hospital wards are shown in Table 2. Evaluation of the distribution of GPB and GNB isolates in different hospital wards has shown that most clinical isolates (25.9% and 24.7%, respectively) were identified in the infection ward.

Pathogen distribution

GNB and GPB comprised 55% (n=622) and 45% (n=508) of the total bacteria, respectively. The isolated GNB included *P. aeruginosa* (n=282; 45.3%), *Klebsiella* spp. (n=100; 16.07%), *Acinetobacter baumannii* (n=83; 13.3%), *E. coli* (n=59; 9.4%), *Enterobacter* spp. (n=45; 7.2%), *Citrobacter* spp. (n=28; 4.5%), *Burkholderia* spp.

(n=20; 3.2%) and *Proteus* spp. (n=5; 0.8%). Moreover, the most predominant isolated GPB was coagulase-negative *Staphylococcus* (CoNS) (n=368; 72.4%), *Enterococcus* spp. (n=71; 13.9%) and *S. aureus* (n=69; 13.5%). The highest number (n=311) of strains was isolated in 2016 and the lowest (n=85) in 2013. Moreover among GNB and GPB, *P. aeruginosa* and CoNS were the most frequent pathogens, respectively (Table 1).

Antimicrobial susceptibility

Resistance rates of GPB to antimicrobials

The resistance rates of the isolated GPB to commonly used antimicrobials are shown in Table 2. In *S. aureus*, isolated from different specimens, the highest resistance rates belonged to oxacillin (n=43/64; 67.2%). However, *S. aureus* was found to have low levels of resistance to vancomycin (n=1/67; 1.5%) and linezolid (n=3/66; 4.5%). In addition, CoNS strains showed a high level of resistance to oxacillin (n=273/318; 85.8%) and ampicillin (n=37/46; 80.4%). vancomycin (n=13/360; 3.6%) and linezolid (n=15/322; 4.7%) were the most effective antimicrobial agents on CoNS.

Enterococcus spp. was 100% resistant to ofloxacin (n=11/11;1 00%), clarithromycin (n=8/8; 100%), cefotaxime (n=3/3;100%), nitrofurantoin (n=11/11; 100%), amikacin (n=11/11; 100%), azithromycin (n=5/5; 100%), clindamycin (n=3/3; 100%), piperacillin/tazobactam (n=2/2; 100%), and doxycycline (n=1/1; 100%) which showed high levels of resistance to trimethoprim-sulfamethoxazole (n=13/14; 92.9%) and quinupristin-dalfopristin (n=10/11; 90.9%). However, *Enterococcus* spp. was found to have low levels of resistance (n=4/59; 6.8%) to linezolid.

Resistance rates of GNB to antimicrobials

Overall, among GNB, *P. aeruginosa* was 100% resistant to amoxicillin-clavulanic acid (n=26/26; 100%) and ticarcillin-clavulanic acid (n=17/17; 100%) and showed a high level of resistance to cefazolin (n=45/46; 97.8%) and ceftriaxone (n=29/31; 93.5%). *P. aeruginosa* was found to have low levels of resistance to levofloxacin (n=20/154; 13%) and ciprofloxacin (n=43/257; 16.7%), respectively. However, 29.8% (n=84/282) of *P. aeruginosa* was MDR.

Levofloxacin (n=19/49; 38.8%) was the most effective antimicrobial agents on *A. baumannii*. However, the resistance level to cefuroxime (n=26/26; 100%), cefotaxime (n=35/36; 97.2%) and meropenem (n=64/68; 94.1%) was high. Moreover, 62.7% (n=52/83) of *A. baumannii* was MDR.

Table I The characteristic of clinical specimens and isolated bacteria in 2013–2018

Gram-positive & negative bacteria	N (male/female) percentage	Hospital wards (N)	Clinical samples (N)
S. aureus	69 (40/29) 58–42%	Hematology (7), Emergency (7), Gastroenterology (7), ICU (1), Infectious (23), Nephrology (3), Neurology (1), NICU(1), Renal unit (1), PICU(14), Rheumatology (1), Surgery (3)	Blood (60), CSF (3), Pleural fluid (4), Dialysis fluid (1), Luminal sample (1)
CoNS	368 (220/148) 59.8–40.2%	Hematology (66), Emergency (17), Gastroenterology (26), ICU (7), Oncology (4), Infectious (92), Nephrology (12), Neurology (20), NICU (10), Urology (2), Renal unit (6), PICU (77), Rheumatology (7), Surgery (22)	Blood (326), CSF (30), Shunt (4), Pleural fluid (8)
Enterococcus spp.	71 (39/32) 54.9–45.1%	PICU (13), Hematology (12), Emergency (2), Gastroenterology (6), ICU (1), Oncology (2), Infectious (17), Neurology (1), NICU (4), Renal unit (2), Rheumatology (1), Surgery (10)	Blood (60), CSF(8), Shunt (1), Pleural fluid (1), Luminal sample (1)
P. aeruginosa	282 (163/119) 57.8–42.2%	Hematology (24), Emergency (10), Gastroenterology (52), ICU (7), Infectious(73), Nephrology (8), Neurology (3), N ICU (3), Renal unit (6), PICU (59), Rheumatology (2), Surgery (35)	Blood (254), CSF (7), Pleural fluid (14), Shunt (1), Luminal sample (6)
A.baumanni	83 (41/42) 49.4–50.6%	Hematology (6), Emergency (1), Gastroenterology (10), Infectious (12), Nephrology (1), Neurology (3), NICU (9), PICU (30), Rheumatology (3), Surgery (8)	Blood (72), CSF (3), Pleural fluid (8)
Klebsiella spp.	100 (62/38) 62–38%	Hematology (18), Emergency (2), Gastroenterology (12), ICU (3), PICU (18), Oncology (1), Infectious (25), Nephrology (4), Neurology (2), NICU (6), Renal unit (2), Surgery (7)	Blood (91), CSF (3), Pleural fluid (6)
E. coli	59 (32/27) 54.2–45.8%	Hematology (11), Gastroenterology (6), ICU (2), Oncology (2), Infectious (21), Nephrology (2), Neurology (3), NICU (2), PICU (10)	Blood (53), CSF (3), Shunt (1), Pleural fluid (2)
Citrobacter spp.	28 (9/19) 32.1–67.9%	Hematology (3), Emergency (1), Gastroenterology (5), Infectious (9), Nephrology (1), Neurology (1), NICU (1), PICU (3), Surgery (4)	Blood (28)
Proteus spp.	5 (3/2) 60–40%	Infectious (2), Emergency (1), Surgery (1), Rheumatology (1)	Blood (4), CSF (1)
Burkholderia. spp.	20 (14/6) 70–30%	Gastroenterology (6), Infectious (1), Nephrology (1), PICU (8), Surgery (4)	Blood (19), Pleural fluid (1)
Enterobacter spp.	45 (18/27) 40–60%	Hematology (4), Emergency (2), Gastroenterology (9), ICU (1), Oncology (1), Infectious (1 2), Nephrology (1), Neurology (1), Renal unit (1), PICU (10), Surgery (3)	Blood (36), CSF (3), Pleural fluid (2), Shunt (4)
Total	1130 (641/489) 56.7–43.7	Hematology (151), Emergency (43), Gastroenterology (139), ICU (22), Infectious (287), Nephrology (33), Neurology (35), NICU (36), Renal unit (14), PICU (242), Rheumatology (15), Surgery (97), Oncology (10), Urology(2)	Blood (1003), CSF (61), Pleural fluid (46), Dialysis fluid (1), Luminal sample(8), Shunt (11)

Table 2 Antimicrobial resistance of isolated GPB from clinical specimens in 2013–2018

Antibiotics		*Staphylococcus aureus*		CoNS		*Enterococcus* spp.	
Penicillins	AMP	S (-)	–	S (19.6%)	9/46	S (15.3%)	9/59
		R (-)	–	R (80.4%)	37/46	R (84.7%)	50/59
	AUG	S (-)	–	S (22.2%)	16/72	S (-)	–
		R (-)	–	R (77.8%)	56/72	R (-)	–
	OX	S (32.8%)	21/64	S (14.2%)	45/318	S (-)	–
		R (67.2%)	43/64	R (85.8%)	273/318	R (-)	–
	PEN	S (-)	–	S (38.3%)	18/47	S (15.8%)	3/19
		R (-)	–	R (61.7%)	29/47	R (84.2%)	16/19
	PTZ	S (-)	–	S (44.7%)	17/38	S (0%)	0/2
		R (-)	–	R (55.3%)	21/38	R (100%)	2/2
	SAM	S (-)	–	S (46.5%)	53/114	S (10%)	1/10
		R (-)	–-	R (53.5%)	61/114	R (90%)	9/10
Fluoroquinolones	LEV	S (-)	–	S (48.4%)	30/62	S (36.4%)	4/11
		R	–	R (51.6%)	32/62	R (63.6%)	7/11
	OFX	S (-)	–	S (43.6%)	17/39	S (0%)	0/11
		R (-)	–-	R (56.4%)	22/39	R (100%)	11/11
	CIP	S (63.8%)	44/69	S (46.6%)	159/341	S (30%)	3/10
		R (36.2%)	25/69	R (53.4%)	182/341	R (70%)	7/10
Macrolides	CLa	S (-)	–	S (-)	–	S (0%)	0/8
		R (-)	–	R (-)	–	R (100%)	8/8
	AZM	S (50%)	19/38	S (-)	–	S (0%)	0/5
		R (50%)	19/38	R (-)	–	R (100%)	5/5
	ERY	S (-)	–	S (20.3%)	26/128	S (17.6%)	3/17
		R (-)	–	R (79.7%)	102/128	R (82.4%)	14/17
Cephalosporins	CTX	S (-)	–	S (-)	–	S (0%)	0/3
		R (-)	–	R (-)	–	R (100%)	3/3
	CRO	S (-)	–	S (-)	–	S (12.5%)	1/8
		R (-)	–	R (-)	–	R (87.5%)	7/8
	CHL	S (83.6%)	46/55	S (70%)	119/170	S (60%)	12/20
		R (16.4%)	9/55	R (30%)	51/170	R (40%)	8/20
Sulfonamides	SXT	S (41.5%)	24/41	S (24%)	25/104	S (7.1%)	1/14
		R (58.5%)	17/41	R (76%)	79/104	R (92.9%)	13/14

(*Continued*)

Table 2 (Continued).

Antibiotics		Isolated bacteria					
		Staphylococcus aureus		CoNS		*Enterococcus* spp.	
Aminoglycosides	GM	S (60.9%) R (39.1%)	39/64 25/64	S (51.3%) R (48.7%)	162/316 154/316	S (18.6%) R (71.4%)	8/28 20/28
	AK	S (-) R (-)	– –	S (79.4%) R (20.6%)	54/68 14/68	S (0%) R (100%)	0/11 11/11
Glycopeptide	VA	S (98.5%) R (1.5%)	66/67 1/67	S (96.4%) R (3.6%)	347/360 13/360	S (46.4%) R (53.6%)	32/69 37/69
Nitrofurans	NI	S (-) R (-)	– –	S (-) R (-)	– –	S (0%) R (100%)	0/11 11/11
Other	RP	S (83.3%) R (16.7%)	35/42 7/42	S (68.6%) R (31.4%)	194/283 89/283	S (9.1%) R (90.9%)	1/11 10/11
Oxazolidinones	LZV	S (95.5%) R (4.5%)	63/66 3/66	S (95.3%) R (4.7%)	307/322 15/322	S (93.2%) R (6.8%)	55/59 4/59
Lincosamides	CLI	S (51.9%) R (48.1%)	28/54 26/54	S (33.6%) R (66.4%)	97/289 192/289	S (0%) R (100%)	0/3 3/3
Tetracyclines	DOX	S (66.6%) R (33.3%)	26/39 13/39	S (62.3%) R (37.7%)	149/239 90/239	S (0%) R (100%)	0/1 1/1

Abbreviations: CIP, ciprofloxacin; GM, gentamicin; PTZ, piperacillin/tazobactam; TET, tetracycline; CHL, chloramphenicol; AK, amikacin; CTX, cefotaxime; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline; MRP, meropenem; DOR, doripenem; IPM, imipenem; SAM, ampicillin-sulbactam; CRO, ceftriaxone; AMP, ampicillin; AUG, amoxicillin-clavulanic acid; NI, nitrofurantoin; AZM, azithromycin; ERY, erythromycin; RP, quinupristin-dalfopristin; VA, vancomycin; CLI, clindamycin; OFX, ofloxacin; LZD, linezolid; PEN, penicillin; CLa, clarithromycin; TIM, ticarcillin-clavulanic acid; R, resistance; S, susceptible; CoNS, coagulase-negative *Staphylococcus*.

The resistance rates of *Klebsiella* spp. to levofloxacin, imipenem and ciprofloxacin were 12.8%, 18.5% and 21%, respectively. In addition, a high level of resistance to cefpodoxime (n=6/6; 100%), ampicillin (n=61/65; 93.8%) and cefazolin (n=39/44; 88.6%) was detected; 27% (n=27/100) of *Klebsiella* spp. strains were MDR.

All of the tested isolates of *Enterobacter* spp. showed resistance to ampicillin (n=23/23; 100%) and tetracycline (n=14/14; 100%) and showed a high level of resistance to ampicillin-sulbactam (n=29/31; 93.5%). Levofloxacin (n=2/12; 16.7%), imipenem (n=7/38; 18.4%) and nalidixic acid (n=1/5; 20%) were the most effective antimicrobial agents on *Enterobacter* spp. Moreover, 26.7% (n=12/45) of *Enterobacter* spp. were MDR.

Proteus spp. was found to have low levels of resistance to imipenem (n=0/5; 0%), ciprofloxacin (n=0/3; 0%), amikacin (n=0/2; 0%) and levofloxacin (n=0/2; 0%); and 20% (n=1/5) of *Proteus* spp. strains were MDR.

For *E. coli*, the resistance rate was 94.7% (n=18/19) for trimethoprim sulfamethoxazole; 94.6% (n=35/37) for ampicillin; 92% (n=23/25) for amoxicillin-clavulanic acid and >80% for several antibiotics such as ceftriaxone, ampicillin-sulbactam, cefuroxime and aztreonam. A high proportion of *E. coli* strains (n=30/59; 50.8%) was MDR.

The resistance rates of *Burkholderia* spp. to trimethoprim-sulfamethoxazole, cefotaxime and ticarcillin-clavulanic acid were 100%, 88.9% and 87.5%, respectively. Ciprofloxacin (n=4/20; 20%), levofloxacin (n=3/12; 25%) and colistin (n=4/15; 26.7%) were the most active antibiotics against *Burkholderia* spp, and 30% (n=6/20) were MDR.

Ciprofloxacin and levofloxacin were the most active antibiotic against *Citrobacter* spp. The resistance level of this organism to ampicillin (n=24/24) and cefazolin (n=7/7) was 100%, and 64.3% (n=18/28). The resistance rates of each GNB to commonly used antimicrobials are shown in Table 3.

Table 3 Antimicrobial resistance of isolated GNB from clinical specimens in 2013–2018

Antibiotics			Isolated bacteria							
			P. aeruginosa	A. baumannii	Burkholderia spp.	E. coli	Klebsiella spp.	Enterobacter spp.	Citrobacter spp.	Proteus spp.
Penicillins	PTZ	S	S (59.5%) 144/242	S (37.8%) 28/74	S (54.5%) 6/11	S (74%) 37/50	S (71.4%) 60/84	S (69.4%) 25/36	S (71.4%) 20/28	S (60%) 3/5
		R	R (40.5%) 98/242	R (62.2%) 46/74	R (45.5%) 5/11	R (26%) 13/50	R (28.6%) 24/84	R (30.6%) 11/36	R (28.6%) 8/28	R (40%) 2/5
	AUG	S	S (0%) 0/26	S (12%) 3/25	S (-) -	S (8%) 2/25	S (12.5%) 6/48	S (10.5%) 2/19	S (3.8%) 1/26	S (0%) 0/3
		R	R (100%) 26/26	R (88%) 22/25	R (-) -	R (92%) 23/25	R (87.5%) 42/48	R (89.5%) 17/19	R (96.2%) 25/26	R (100%) 3/3
	AMP	S	S (-) -	S (9.1%) 3/33	S (-) -	S (5.4%) 2/37	S (6.2%) 4/65	S (0%) 0/23	S (0%) 0/24	S (25%) 1/4
		R	R (-) -	R (90.9%) 30/33	R (-) -	R (94.6%) 35/37	R (93.8%) 61/65	R (100%) 23/23	R (100%) 24/24	R (75%) 3/4
	SAM	S	S (16.7%) 12/72	S (18.7%) 12/64	S (0%) 0/1	S (13.3%) 6/45	S (13.3%) 11/83	S (6.5%) 2/31	S (23.1%) 6/26	S (40%) 2/5
		R	R (83.3%) 60/72	R (81.3%) 52/64	R (100%) 1/1	R (86.7%) 39/45	R (86.7%) 72/83	R (93.5%) 29/31	R (76.9%) 20/26	R (60%) 3/5
	TIM	S	S (0%) 0/17	S (-) -	S (22.5%) 1/8	S (-) -	S (-) -	S (-) -	S (-) -	S (-) -
		R	R (100%) 17/17	R (-) -	R (87.5%) 7/8	R (-) -	R (-) -	R (-) -	R (-) -	R (-) -
Enems	MRP	S	S (29.7%) 63/212	S (5.9%) 4/68	S (40%) 6/15	S (65.9%) 27/41	SR 42/74	S (48.7%) 19/39	S (23.1%) 6/26	S (50%) 2/4
		R	R (70.3%) 149/212	R (94.1%) 64/68	R (60%) 9/15	R (34.1%) 14/41	(43.2%) 32/74	R (51.3%) 20/39	R (76.9%) 20/26	R (50%) 2/4
	IPM	S	S (49.6%) 126/254	S (17.3%) 13/75	S (35%) 7/20	S (76.1%) 35/46	S (81.5%) 66/81	S (81.6%) 31/38	S (46.4%) 13/28	S (100%) 5/5
		R	R (50.4%) 128/254	R (82.7%) 62/75	R (65%) 13/20	R (23.9%) 11/46	R (18.5%) 15/81	R (18.4%) 7/38	R (53.6%) 15/28	R (0%) 0/5
Cephalosporins	CAZ	S	S (33.9%) 86/254	S (26.2%) 21/80	S (47.4%) 9/19	S (23.2%) 13/56	S (33%) 30/91	S (30%) 12/40	S (25.9%) 7/27	S (66.6%) 2/3
		R	R (66.1%) 168/254	R (73.8%) 59/80	R (52.6%) 10/19	R (76.8%) 43/56	R (67%) 61/91	R (70%) 28/40	R (74.1%) 20/27	R (33.3%) 1/3
	CRO	S	S (6.5%) 2/31	S (7.7%) 3/39	S (-) -	S (15%) 3/20	S (18.2%) 2/11	S (33.3%) 6/18	S (24%) 6/25	S (0%) 0/3
		R	R (93.5%) 29/31	R (92.3%) 36/39	R (-) -	R (85%) 17/20	R (81.8%) 9/11	R (66.7%) 12/18	R (76%) 19/25	R (100%) 3/3
	CPd	S	S (-) -	S (-) -	S (-) -	S (-) -	S (0%) 0/6	S (-) -	S (-) -	S (-) -
		R	R (-) -	R (-) -	R (-) -	R (-) -	R (100%) 6/6	R (-) -	R (-) -	R (-) -
	CZ	S	S (2.2%) 1/46	S (0%) 0/26	S (-) -	S (22.2%) 4/18	S (11.4%) 8/4	S (11.8%) 2/17	S (0%) 0/7	S (-) -
		R	R (97.8%) 45/46	R (100%) 26/26	R (-) -	R (77.8%) 14/18	R (88.6%) 436/44	R (88.2%) 15/17	R (100%) 7/7	R (-) -
	CXM	S	S (15.2%) 7/46	S (0%) 0/26	S (-) -	S (15.4%) 4/26	S (28.3%) 13/46	S (10.5%) 2/19	S (0%) 0/33/3	S (0%) 0/1
		R	R (84.8%) 39/46	R (100%) 26/26	R (-) -	R (84.6%) 22/26	R (71.7%) 33/46	R (89.5%) 17/19	R (100%)	R (100%) 1/1
	CTX	S	S (10.9%) 7/64	S (2.8%) 1/36	S (11.1%) 1/9	S (19.2%) 5/26	S (32.7%) 16/49	S (15.4%) 4/26	S (0%) 0/2	S (-) -
		R	R (89.1%) 57/64	R (97.2%) 35/36	R (88.9%) 8/9	R (80.8%) 21/26	R (67.3%) 33/49	R (84.6%) 22/26	R (100%) 2/2	R (-) -
	CPM	S	S (17.1%) 29/170	S (21.4%) 15/70	S (-) -	S (34.3%) 12/35	S (47.1%) 33/70	S (28.6%) 10/35	S (-) -	S (-) -
		R	R (82.9%) 141/170	R (78.6%) 55/70	R (-) -	R (65.7%) 23/35	R (52.9%) 37/70	R (71.4%) 25/35	R (-) -	R (-) -
Fluoroquinolones	CIP	S	S (83.3%) 214/257	S (53.3%) 40/75	S (80%) 16/20	S (53.1%) 26/49	S (79%) 64/81	S (78.9%) 30/38	S (83.3%) 10/12	S (100%) 3/3
		R	R (16.7%) 43/257	R (46.7%) 35/75	R (20%) 4/20	R (46.9%) 23/49	R (21%) 17/81	R (21.1%) 8/38	R (16.7%) 2/12	R (0%) 0/3
	LEV	S	S (87%) 134/154	S (61.2%) 30/49	S (75%) 9/12	S (56.2%) 9/16	S (87.2%) 34/39	S (83.3%) 10/12	S (83.3%) 10/12	S (100%) 2/2
		R	R (13%) 20/154	R (38.8%) 19/49	R (25%) 3/12	R (43.8%) 7/16	R (12.8%) 5/39	R (16.7%) 2/12	R (16.7%) 2/12	R (0%) 0/2
	NA	S	S (40%) 2/5	S (-) -	S (-) -	S (83.3%) 5/6	S (58.3%) 7/12	S (80%) 4/5	S (68.2%) 13/19	S (50%) 1/2
		R	R (60%) 3/5	R (-) -	R (-) -	R (16.7%) 1/6	R (41.7%) 5/12	R (20%) 1/5	R (31.6%) 6/19	R (50%) 1/2

(Continued)

Table 3 (Continued).

Antibiotics		S/R	Isolated bacteria							
			P. aeruginosa	A. baumannii	Burkholderia spp.	E. coli	Klebsiella spp.	Enterobacter spp.	Citrobacter spp.	Proteus spp.
Aminoglycosides	GM	S	(41.9%) 104/248	(48.7%) 38/78	(31.6%) 6/19	(51.9%) 27/52	(45.6%) 41/90	(40.5%) 17/42	(46.4%) 28	(75%) 3/4
		R	(58.1%) 144/248	(51.3%) 40/78	(68.4%) 13/19	(48.1%) 25/52	(54.4%) 49/90	(59.5%) 25/42	(53.6%) 15/28	(25%) 1/4
	AK	S	(56.8%) 125/	(50.7%) 35/69	(-) –	(78.6%) 33/42	(65.8%) 52/79	(66.6%) 18/27	(47.8%) 11/23	(100%) 2/2
		R	(43.2%) 95/220	(49.3%) 34/69	(-) –	(21.4%) 9/42	(34.2%) 27/79	(33.3%) 9/27	(52.2%) 12/23	(0%) 0/2
	TOB	S	(34.5%) 57/165	(33.3%) 16/48	(50%) 8/16	(52.9%) 9/17	(51.7%) 15/29	(20%) 4/20	(0%) 0/2	(50%) 1/2
		R	(65.5%) 108/165	(66.7%) 32/48	(50%) 8/16	(47.1%) 8/17	(48.3%) 14/29	(80%) 16/20	(100%) 2/2	(50%) 1/2
Tetracyclines	TET	S	(35.7%) 5/14	(42.9%) 3/7	(-) –	–	(68.7%) 11/16	(0%) 0/3	(37.5%) 3/8	(0%) 0/1
		R	(64.3%) 9/14	(57.1%) 4/7	(-) –	–	(31.3%) 5/16	(100%) 3/3	(62.5%) 5/8	(100%) 1/1
Other	PB	S	(71.4%) 5/7	(-) –	(-) –	(-) –	(66.6%) 2/3	(-) –	(-) –	(-) –
		R	(28.6%) 2/7	(-) –	(-) –	(-) –	(33.3%) 1/3	(-) –	(-) –	(-) –
	CL	S	(59.8%) 143/239	(50%) 22/44	(73.3%) 9/15	(71.8%) 28/39	(-) –	(74.4%) 29/39	(63.6%) 7/11	(-) –
		R	(40.2%) 96/239	(50%) 22/44	(26.7%) 4/15	(28.2%) 11/39	(-) –	(25.6%) 10/39	(36.4%) 4/11	(-) –
Macrolides	AZM	S	(-) –	(-) –	(-) –	(41.2%) 7/17	(-) –	(-) –	(-) –	(-) –
		R	(-) –	(-) –	(-) –	(58.8%) 10/17	(-) –	(-) –	(-) –	(-) –
Nitrofurans	NI	S	(-) –	(-) –	(-) –	(40%) 2/5	(25%) 1/4	(0%) 0/3	(17.4%) 4/23	(0%) 0/2
		R	(-) –	(-) –	(-) –	(60%) 3/5	(75%) 3/4	(100%) 3/3	(82.6%) 19/23	(100%) 2/2
Sulfonamides	SXT	S	(48.8%) 21/43	(48.1%) 13/27	(57.1%) 8/14	(5.3%) 1/19	(36.4%) 15/41	(53.8%) 7/13	(50%) 3/6	(-) –
		R	(51.2%) 22/43	(51.9%) 14/27	(42.9%) 6/14	(94.7%) 18/19	(63.4%) 26/41	(46.2%) 6/13	(50%) 3/6	(-) –
Monobactams	ATM	S	(17.3%) 27/156	(-) –	(22.2%) 2/9	–	(28.6%) 6/21	(75%) 3/4	(-) –	(0%) 0/1
		R	(82.7%) 129/156	(-) –	(77.8%) 7/9	–	(71.4%) 15/21	(25%) 1/4	(-) –	(100%) 1/1

Abbreviations: CIP, ciprofloxacin; PTZ, piperacillin/tazobactam; TET, tetracycline; AK, amikacin; CTX, cefotaxime; CAZ, ceftazidime; CPM, cefepime; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; TOB, tobramycin; MRP, meropenem; IPM, imipenem; SAM, ampicillin-sulbactam; CRO, ceftriaxone; CXM, cefuroxime; AMP, ampicillin; AUG, amoxicillin-clavulanic acid; CL, colistin; NI, nitrofurantoin; GM, gentamicin; NA, nalidixic; CZ, cefazolin; AZM, azithromycin; PB, polymyxin B; CPd, cefpodoxime; TIM, ticarcillin-clavulanic acid; ATM, aztreonam.

Time trends in antibiotic resistance among GPB and GNB isolated from Clinical specimens in 2013–2018

The time trend analysis of antibiotic resistance among GPB and GNB is shown in Tables 4 and 5, respectively. Among GPB, results show that S. aureus isolates had the highest resistance rate to linezolid (R=50%) in 2017. Moreover, vancomycin is an effective antibiotics against GPB and has a lowest resistance rate (R=4.5% in 2017) against S. aureus isolates.

In 2015, Enterococcus isolates had the highest resistance to vancomycin (R=63.6%), and levofloxacin was the most effective antibiotic against Enterococcus isolates in 2017 (resistance has not been seen). In CoNS isolates, the highest and lowest resistance to vancomycin was seen in 2013 and 2018, respectively. Linezolid was an effective antibiotic against CoNS isolates in 2013 (resistance has not been seen) and the highest resistance was seen (R=15.4%) in 2014. Among GNB, levofloxacin was the most effective antibiotic against P. aeruginosa isolates in 2013 and resistance has not been seen. However, in 2014

levofloxacin has the highest resistance (R=50%) against this pathogen. In P. aeruginosa isolates, ciprofloxacin has the highest and lowest resistance in 2013 and 2016, respectively. Our analysis showed that A. baumannii has the highest resistance to levofloxacin (R=80%) in 2018.

Discussion

This study examined the prevalence of antibiotic resistance among main pathogenic bacteria isolated from hospitalized children in Mofid Children's Hospital, Tehran, Iran. Given that these antibiotic resistance for GNB and GPB can cause severe infections in hospitalized patients, especially in neonates and children, the presence and distribution of these agents is one of the main concerns for physicians.[23,24] Since the application of several categories of antibiotics is not permissible in neonates and children and since there are different patterns of AMR in various areas, selecting and prescribing appropriate antibiotics for the treatment of various infections in pediatric patients is challenging.[15,25] Moreover, knowing AMR patterns can

Table 4 Trends in antibiotic resistance among GPB isolated from clinical specimens in 2013–2018

Time						
Antibiotics/organism	2013	2014	2015	2016	2017	2018
S. aureus						
VA	-	(0%)	0%	0%	4.5%	0%
LZV	-	(0%)	50%	0%	9.5%	0%
GM	-	50%	50%	28.65	36.4%	25%
CIP	-	37.5%	-40%	21.4%	45.55	20%
DOX	-	-	-	38.5%	28.6%	40%
Enterococcus spp.						
VA	50%	50%	63.6%	52.4%	50%	57.1%
LZV	0%	33.3%	22.2%	0%	0%	0%
GM	100%	505	63.6%	-	-	100%
CIP	-	-	83.3%	50%	-	-
AMP	100%	75%	80%	85.7%	92.9%	75%
LEV	66.7%	75%	-	-	0%	-
CoNS						
OX	85.3%	86.4%	95.7%	81.1%	84.3%	86.8%
SAM	43.2%	74.2%	45.5%	-	100%	100%
CIP	45.9%	58.5%	45.5%	51.4%	54.3%	62.3%
SXT	58.3%	91.7%	77.3%	50%	63.3%	100%
ERY	77.8%	76.2%	86%	100%	60%	-
GM	53.1%	60.6%	36.4%	41.3%	50.5%	59.5%
VA	8.3%	2.3%	2.1%	5.4%	4%	0%
RP	22.2%	53.3%	-	30.1%	30.8%	28.3%
LZV	0%	15.4%	2.1%	6.6%	3.2%	3.4%
CLI	46.4%	61.1%	67.9%	67.1%	71.2%	72.9%
DOX	50%	66.7%	-	29.1%	39.8%	41.2%

Table 5 Trends in antibiotic resistance among GNB isolated from clinical specimens in 2013–2018

Time						
Antibiotics/ organisms	2013	2014	2015	2016	2017	2018
P. aeruginosa						
PTZ	25%	28.6%	30.8%	33.8%	45.9%	60.5%
MRP	75%	100%	50%	71.8%	54.1%	78.9%
IPM	37.5%	42.9%	30%	56.6%	50%	66.7%
CAZ	50%	89.3%	91.9%	75.9%	35.6%	55.8%
CPM	-	-	-	86.5%	73.7%	89.7%
CIP	25%	21.4%	23.7%	11.4%	15.3%	17.8%
LEV	0%	50%	-	13.9%	14%	8%
GM	37.5%	80%	80.6%	64.6%	43.8%	31.8%
AK	50%	100%	66.7%	47.6%	25.5%	23.7%
CL	28.6%	55.2%	31.4%	53.8%	36.2%	12.55
ATM	-	-	-	87.9%	71.45	87.8%
CRO	0%	100%	100%	94.1%	100%	-
A. baumannii						
PTZ	-	25%	100%	34.6%	69.25	100%
SAM	-	100%	100%	72.7%	73.9%	100%
MRP	-	100%	100%	92.3%	92.3%	100%
IPM	-	60%	100%	70.8%	84%	100%
CAZ	-	83.3%	66.7%	55.6%	79.2%	100%
CRO	-	100%	100%	100%	82.4%	100%
CPM	-	100%	100%	60.7%	80%	100%
CIP	-	33.3%	57.1%	25.9%	53.8%	87.5%
LEV	-	0%	0%	25%	53.35	80%
GM	-	50%	55.6%	26.9%	57.7%	90.9%
AK	-	66.7%	50%	26.1%	48%	90.9%
CL	-	-	-	-	26.75	80%
E. coli						
PTZ	40%	100%	20%	16.7%	29.4%	42.9%
AMP	-	-	100%	66.7%	100%	-
SAM	100%	100%	100%	83.3%	73.3%	80%
MRP	60%	25%	50%	33.3%	25%	33.3%
IPM	60%	0%	40%	0%	16.7%	42.9%
CAZ	80%	62.5%	100%	100%	63.2%	71.4%
CXM	100%	85.7%	-	100%	77.8%	75%
CTX	-	-	100%	100%	60%	75%
CPM	-	-	57.1%	60%	64.7%	83.3%
CIP	-	80%	50%	50%	33.3%	28.6%
LEV	-	100%	100%	66.7%	33.3%	0%
GM	60%	12.5%	30%	66.7%	64.7%	50%
AK	40%	100%	0%	33.3%	28.6%	25%
CL	-	22.2%	50%	0%	27.8%	60%
Klebsiella spp.						
PTZ	50%	33.3%	40%	3.4%	52.9%	27.3%
AMP	-	100%	83.3%	50%	-	-

(Continued)

Table 5 (Continued).

Time						
Antibiotics/ organisms	2013	2014	2015	2016	2017	2018
SAM	100%	100%	88.9%	80%	82.4%	83.3%
MRP	62.5%	80%	100%	43.3%	27.8%	27.3%
IPM	25%	22.2%	12.5%	12.5%	21.1%	23.1%
CAZ	85.7%	72.7%	76.9%	50%	77.8%	64.3%
CTX	-	85.75	63.6%	33.3%	91.7%	53.8%
CPM	-	50%	54.5%	35.7%	87.5%	46.2%
CIP	40%	10%	27.3%	6.9%	50%	16.7%
LEV	20%	20%	0%	0%	57.1%	0%
GM	42.9%	58.3%	30%	40%	88.2%	64.3%
AK	66.7%	54.5%	30%	14.3%	64.3%	10%
Enterobacter spp.						
PTZ	0%	66.7%	33.3%	100%	23.1%	30%
AMP	100%	100%	-	100%	100%	100%
SAM	80%	100%	100%	100%	91.7%	100%
MRP	40%	60%	75%	-	50%	45.5%
IMP	0%	60%	20%	0%	28.6%	25%
CAZ	0%	0%	42.9%	100%	78.6%	100%
CTX	-	83.35	50%	-	100%	100%
CPM	-	60%	33.3%	100%	84.6%	80%
CIP	0%	16.7%	50%	50%	28.6%	0%
LEV	0%	0%	0%	-	20%	50%
GM	0%	66.7%	42.9%	100%	78.6%	62.5%
AK	-	-	40%	-	23.1%	44.4%
CL	0%	0%	50%	100%	27.3%	30%

help physicians and policy makers to find solutions for resistance problems in their countries.[23,26] Lack of general AMR surveillance programs in developing and several developed countries will lead to inappropriate use among patients and health care staff.[27] Therefore, investigating AMR patterns are very critical and important, mainly in developing countries such as Iran, where there is no systematic guidelines for antibiotic usage. On the other hand, it is necessary to analyze the patterns of antibiotic resistance for GPB and GNB at Mofid Children's Hospital of Tehran, capital of Iran, during 2013–2018, which can be a valuable model for both clinicians and policy makers in implementing empirical therapy. The result of our study revealed that among 14,690 different clinical samples from unique patients, 1130 (7.7%) cultures were positive from which various bacteria were isolated. The low rate of positive culture in the current study can be due to several reasons: 1) our study used various types of clinical samples such as blood, CSF, pleural fluid, dialysis fluid and

luminal fluid in which the rate of pathogens in these specimens is different, 2) effective training for the correct administration of antibiotic, 3) better management and control of infection, and 4) pre-hospitalization use of antibiotics. The amount of GNB and GPB isolates among all positive cultures were 55% (n=622) and 45% (n=508), respectively, and it was detected that GNBs are frequently isolated in positive cultures. In the present study, *P. aeruginosa* and CoNS were the most frequent pathogens among GNB and GPB, respectively, which is in agreement with a study conducted by Mahmoudi et al (2011–2016 Tehran, Iran).[12] However, in the investigations carried out by Ebrahim-Saraie et al and Alam et al, *Acinetobacter* spp. was the most common GNB in positive culture specimens.[28,29] The result of a published study revealed that *E. coli* was the most frequent Gram-negative pathogen in positive cultures of the specimens[30]. The detected differences in proportions of GNB and GPB could be due to the diversity of specimen type, specimen size and applied detecting methods. Among different antibiotics that were tested against *P. aeruginosa*, levofloxacin and ciprofloxacin were the effective antibiotics, respectively. On the other hand, *P. aeruginosa* showed a high level of resistance to ticarcillin-clavulanic acid, cefazolin and ceftriaxone. Similarly, *A. baumannii* showed the lowest and highest resistance rate to levofloxacin and meropenem, respectively.

Results of time trend analyses showed that levofloxacin resistance rate against *P. aeruginosa* has decreased from 2014 to 2018. Moreover, these results showed that levofloxacin resistance rate against *A. baumannii* has increased from 2016 to 2018.

According to this date, suitable antibiotic selection is significant and vital in the treatment of bacterial infections. Therefore, awareness regarding antibiotic resistance patterns in pathogenic bacteria can be helpful in making the right therapeutic choice. The results have also shown that probably CoNS isolated from clinical specimens was considered as a common contaminant. Therefore, more effective measures such as hand hygiene of health care workers, regular disinfection of medical devices, and disinfection of sampling site need to be taken during sampling. However, albeit rare, CoNS can cause several infections including skin and soft tissue infections and thus should not at all times be considered as contaminants.[31] Persistent CoNS infection is probably related to various severe complications such as embolic complications, metastatic seeding and septic thrombophlebitis.[32] Therefore, the evaluation of

CoNS medical correlation is a challenging problem. In medical diagnostic laboratories, the main diagnostic challenge is to evaluate whether an expected CoNS isolate represents: 1) a common colonization of the skin, soft tissue or mucous membranes, 2) a contamination of the specimen throughout sample collection, handling and processing, or 3) clinically important infection.[33] In the case of coinfection of CoNS with other bacterial infections (polymicrobial infections by CoNS), different bacteria isolates showed various susceptibility and resistance patterns, this diagnostic challenging situation becomes even extra intricate.[32,33] A close collaboration between medicians and diagnostic laboratory specialists can solve this medical and diagnostic problem. In case of false-positive CoNS cases, the patients are treated with several antibiotics, it is predicted that besides additional costs, excessive antibiotic selection pressures occur which can lead to the emergence of antibiotic resistance.[34] Therefore, it is important to answer the question that CoNS isolated from a clinical specimen is a real infection or only a common contamination or skin colonization. Some of the main factors useful in the prediction of real infection are: 1) similar strains being isolated repeatedly during course of an infection after the isolation of a strain in pure culture from the infected site, 2) in bloodstream infections, patients should have clinical evidence of the infection with one positive blood culture or only two positive CoNS blood cultures within 5 days, and 3) if CoNS is isolated from a skin or soft tissue bacterial culture of a suspected infectious lesion, the isolated organism should be proposed as a pathogen and suitable treatment should be initiated.[35–37]

Among different-tested antibiotics, the results of our study have revealed that linezolid and vancomycin are effective antibiotics against *S. aureus* and *Enterococcus* spp, which was in agreement with the rates reported by Dharmapalan et al from India,[38] He et al from China,[39] Lei Tian et al from China,[40] and Al-Naqshbandi et al from Iraq.[41] However, the results of several studies were not consistent with our research and it has been reported that the resistance to vancomycin is high.[42,43] In the present study, one case of vancomycin resistant to *S. aureus* (VRSA) was observed; however, vancomycin resistance in *Enterococcus* spp. was much higher (37/69); 53.6% of *Enterococcus* spp. isolates were vancomycin-resistant. Although the identification of *Enterococcus* spp. was not performed to species level, we proposed that most vancomycin-resistant isolates are more likely to be Enterococcus *faecium*. According to several published studies and

reports, effective measures were taken to decrease the risk of VRSA in several countries such as the USA, also some guidelines were developed to control the infections caused by these pathogenic microorganisms.[15] Thus, we suggest similar guidelines and programs designed for children and neonates patients in Tehran, Iran. All in all, the results of the current study revealed that ticarcillin-clavulanic acid, ampicillin, amoxicillin-clavulanic acid, cefazolin and ceftriaxone are ineffective antibiotics against GNB. Notably, in different hospitals in Tehran, these antibiotics are frequently used to control various infections especially sepsis and septicemia. It is well understood that resistance to these antibiotics increases daily, and it is the consequence of selective pressure excreted via bystander selection and abuse or overuse of antibiotics.[44] According to high antibiotic resistance among bacteria, in order to prevent undesirable effects of sepsis and septicemia, as well as in order to reduce the mortality rate due to these infections, precise detection and use of effective antibiotics for an efficient treatment are critical.[8,45–47] Consequently, awareness of the antibiotic resistance patterns among common pathogens, holding workshops to correct prescription of empiric treatment, and changes in antimicrobial use are warranted and highly recommended. Finally, the results of DDM and MIC tests are of great importance, and individuals' free access to antibiotics should be prevented. In this study, we have revealed that GNB and GPB are resistant to various groups of antibiotics; however, it should be noted that these bacteria have two types of antibiotic resistance: acquired resistance and intrinsic resistance. For instance, according to EUCAST guideline, most GNB (*Enterobacteriaceae, Pseudomonas* spp.) have an intrinsic resistance to various antibiotics including penicillin G, oxacillin, macrolides (eg, azithromycin, erythromycin, tylosin), lincosamides (eg, lincomycin, clindamycin), streptogramins (eg, virginiamycin), glycopeptides (eg, vancomycin) and bacitracin. Moreover, based on these guidelines, most GPB are intrinsically resistant to polymyxins and quinolones/fluoroquinolones (eg, enrofloxacin, ciprofloxacin, difloxacin, marbofloxacin).[48] Therefore, these resistances should be known by clinicians in order to avoid unsuitable and ineffective therapy.

Our study also revealed that colistin, in comparison with levofloxacin and ciprofloxacin, has the highest resistance rate. These finding were in contrast with the results of Mahmoudi et al from Iran[12] and Dharmapalan et al from India.[38] In the current study, among GNB, *Proteus* spp. and *Citrobacter* spp. have the lowest and highest rate

of MDR, respectively. The infection with MDR bacteria has several unfortunate consequences such as increased hospitalization, increase in health care and hospitalization costs, reduction in success rate of the infection treatments and increase in morbidity and mortality rates.[12] Moreover, according to results obtained from the prevalence of MDR bacteria that revealed the incidence of MDR bacteria is increasing and by considering the fact that there are limited therapeutic options for MDR bacterial infections, serious measures such as well-controlled clinical trials of combinations of existing antibiotics (for instance colistin plus rifampicin against MDR *P. aeruginosa* infections, colistin plus vancomycin, colistin–carbapenem, trimethoprim-sulfamethoxazole with colistin or fosfomycin and aminoglycoside against enterobacteriaceae infection or combination of cloxacillin (150 mg/kg/day dose), along with gentamicin in CoNS blood infection should be urgently taken.[49] On the other hand, systematic surveillance of hospitals and community-acquired infections, hospital waste management, monitoring the use of antibiotics, monitoring and evaluating antibiotic sensitivity, and preparing reliable antibiotic strategies are necessary.

Our study has several limitations including: 1) it was not a cross-sectional investigation and had a retrospective nature; therefore, given the incompleteness of databases, several main clinical data of patients including results of treatment and mortality rate due to GPB and GNB were not available and we could not include this information in our research, 2) there was no control group for some variables in this study, 3) it also lacked access to patients information such as treatment outcomes and mortality rate due to GNB and GPB, 4) and finally the classification of GNB and GPB, because of their unknown origins (hospital or community-acquired infections) was not performed, 5) the identification of *Citrobacter, Enterococcus*, CoNS, *Enterobacter, Klebsiella, Burkholderia* and *Proteus* were not performed to species level; therefore, determining the infection rates and antibiotic resistance patterns of different species of these bacteria was impossible. 6) Considering several factors including annual changes in hospital policies, availability of antibiotics in laboratory and based on the antibiotic resistance patterns reported by laboratory experts as well as physicians' recommendations, the utilized antimicrobial agents and the number of tested isolates varied from year to year. Consequently, in the case of some antibiotics including imipenem, ceftriaxone, and several other antibiotics, there were few-studied cases; so, their susceptibility and resistance patterns do not

absolutely reflect their situations. Therefore, the applied antibiotics for the treatment of various bacterial infection varied too. There have been few studies on several antibiotics including cefotaxime, ceftriaxone, clarithromycin and nitrofurantoin among GPB, and polymyxin B, cefpodoxime and azithromycin among GNB, and thus their AMR patterns do not fully reflect their resistance or susceptibility positions. 7) Moreover, based on the fact that we did not have full access to patients information such as treatment outcomes, mortality rate, etc., no specific analysis was performed.

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Author contributions

All authors contributed to data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work. Taher Azimi and Saied Maham share first authorship.

Disclosure

The authors report no conflicts of interest in this work.

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