ORIGINAL ARTICLE

Prevalence of Enterobacteriaceae spp. and its multidrug-resistant rates in clinical isolates: A two-center cross-sectional study

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Abstract

Enterobacteriaceae spp., owing to their high durability and antibiotic-resistant mechanisms, are described as an eminent part of health treatments in hospital-acquired infections. This study aimed to estimate the prevalence of clinical isolated Enterobacteriaceae spp., and their multidrug-resistant rate in the north of Iran. In this cross-sectional study, over two years (2017–2019), clinical isolates were collected and Enterobacteriaceae spp. were identified using the standard media culture and Analytical Profile Index (API 20E) kit from two centers in the north of Iran. Isolates were confirmed by targeting the *rpoB* gene. Moreover, the susceptibility patterns of isolates were assessed using disc diffusion methods according to the Clinical Laboratory Standard Institute (CLSI) guidelines. Out of 2645 clinical specimens, 297 (11.2%) were confirmed as Enterobacteriaceae spp. containing *Eshershia. coli* 93 (31%), *Citrobacter freundii* 65 (21.9%), *Klebsiella pneumoniae* 48 (16.2%), Enterobacter spp. 43 (14.5%), and Proteus spp. 23 (7.7%). As much as 8.7% of other spp. Ampicillin (81.1%) and cephalexin (80.9%) have been shown to have the greatest resistant, and nalidixic acid (65%) and amikacin (59.2%) were the most sensitive drugs. Multidrug-resistance (MDR) strains are more isolated in the Burn and Burn intensive care unit (BICU) than other wards. The MDR frequency in Bouali and Zareh hospitals were 65 (49.61%) and 130 (78.31%), respectively. Considering the high isolation rates of MDR Enterobacteriaceae spp., preventive measures need to be taken to remove the mentioned bacteria from hospital wards.

Keywords Enterobacteriaceae · Hospital-acquired infections · Susceptibility testing · Drug-resistant

Background

Enterobacteriaceae is an opportunistic gram-negative bacterial family. Due to their antibiotic resistance, they can survive in hospital settings leading to hospital-acquired infections (HAIs) and higher morbidity and mortality.[1]. These bacteria are present in human and animal gastrointestinal tracts and cause diseases in immunocompromised individuals, burn patients, and patients in intensive care units (ICUs) [2]. *Klebsiella pneumoniae*, *Escherichia coli*, Proteus, and Enterobacter spp. are more isolated than other Enterobacteriaceae spp. from clinical specimens [3]. The most common causes of urinary tract infections (UTIs) in children are bacteria from the Enterobacteriaceae family, such as *E. coli* and Klebsiella spp. Enterobacter, another member of the Enterobacteriaceae family, also has the ability to cause UTI, and respiratory tract, blood, and skin infections in hospital wards[4]. There are major concerns about Citrobacter spp., as it is an infectious agent of meningitis that causes 30% of mortalities and more than 80% of the developmental disorders of surviving patients [5]. The presence of antibiotic resistance sequences, especially carbapenemase-producing genes, is the main reason for the antibiotic resistance in Enterobacteriaceae, which entails prolonged hospitalization and limitations in treatment with antibiotics such as broad-spectrum penicillins, third-generation cephalosporins, fluoroquinolones, and carbapenems [6]. Multi-drug resistant (MDR) and extended-drug resistant (XDR) Enterobacteriaceae are responsible for a variety of infections in humans

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and animals. According to the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), the MDR and XDR are defined as follows: MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories; and XDR: non-susceptible to ≥1 agent in all but ≤2 categories"[7]. Higher prevalence of MDR and XDR strains can prolong the treatment and hospitalization with more expensive medical interventions and elevated risk of mortality. [3]. For instance, more than 90,000 deaths are estimated to have occurred following approximately 1.7 million HAIs in 2019. [6]. In Europe, the annual incidence of HAIs is estimated to be about 4 million cases, while the rate of HAIs in Brazilian hospitals is an estimated 15% [6]. Due to higher level of divergence, RNA polymerase beta-subunit encoding gene (*rpoB*) sequence has more differentiation power than 16 s rRNA for enteric bacteria, and can be used as a molecular method for enteric bacteria identification [8]. Accordingly, regarding the undeniable role of the Enterobacteriaceae in the HAIs and their high antibiotics resistance, the present study evaluated the prevalence of the aforementioned phenotypes and their antibiotic susceptibility patterns.

Methods

Sample collection and bacterial culture

In the current cross-sectional study, a total of 2645 clinical samples including wound, urine, sputum, semen, eye discharge, blood, stool, and pleura fluids were isolated in 2 years (2017–2019) from the two main centers of Bouali (Center 1) and Zareh (Center 2) hospitals, in the north of Iran. The clinical samples were cultured on selective media including Blood agar, MacConkey agar, and Eosin methylene blue agar (EMB) medium. Blood samples were grown on TSB (Tryptic Soy Broth), and following 24–48 h of incubation at 37 °C bacterial identification was performed according to gram staining and the conventional protocols introduced by Bergey [9]. In brief, biochemical tests including oxidase, catalase, motility, Citrate, Indol, Methyl red, Voges–Proskauer, Lysine decarboxylase, and Arginine dehydrogenase as the gold standard test for identification of Enterobacteriaceae were performed in suspected growth colonies.

Reconfirmation of isolated bacteria

Bacterial isolates were precisely identified following a specified phenotypic procedure named the Analytical Profile Index (API 20E) kit (BioMérieux, Marcy l'Etoile, France) using the identification chart supplied by the manufacturer [10].

Molecular approaches

Precise identification of Enterobacteriaceae spp. isolates was accomplished using a pair designed primer to *rpoB* as targeted genes according to a previously published study [11]. Briefly, 512 bp targeted genes were amplified based on the extracted genomic crude DNA of each isolated strain by the boiling procedure as the template in 25 µl defined volume on the Gene Amp PCR system (Applied Biosystem, USA) in a total volume of 25 µl containing 1 µl of DNA, 10 pmol of each forward and reverse primers, 12 µl of Master Mix (Takara, Japan), and 10 µl of double-distilled water. The first cycle of denaturation occurred at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30 s, then at 60 °C for 1 min, at 72 °C for 1 min, and finally a terminal extension for 5 min. The resulting PCR was visualized in a 1% agarose gel (KBC, Max Pure agarose, Spain). *S. epidermidis* ATCC 12228 and *Escherichia coli* ATCC 25922 were used as the negative and positive control, respectively.

Susceptibility testing

The antibiotics susceptibility test on confirmed Enterobacteriaceae isolates was determined by the disc diffusion method under the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. Briefly, a standard 0.5 McFarland suspension was prepared from each isolate and cultured uniformly on the Müller-Hinton agar medium by streaking the swab in back and forth motions. Antimicrobial discs (BD BBLTM Sensi DiscTM) including amikacin (30 µg), ceftazidime (30 µg), cephalexin (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), cefepime (30 µg), ampicillin (10 µg), piperacillin (100 µg), nalidixic acid (30 µg), and nitrofurantoin (300 µg) were placed on the agar plates, and incubated for 24 h at 37 °C. Following the incubation, inhibition zone size was measured using a ruler. Susceptibility or resistance of the organism to each tested drug was determined, using published CLSI guidelines. *E. coli* ATCC 25922 served as a positive control in all phenotypic procedures as well. Moreover, interpretation of Antibiotic susceptibility to evaluated MDR isolates was performed based on the ECDC instructor as previously described [7].

Statistical analysis

The data analysis by SPSS software version 20 was studied. The frequency and percent (%) were shown to reveal the quality variables; and, the means and criteria bias were employed for expressing quantity variables. The Chi-Square test was used for assessing the relationship between qualitative variables. The *P* value less than 0.05 considered a

statistically significant. The categorical data (Sex, hospital wards and resistant patterns) was analyzed using the Fisher Exact test. Contingency at sex, hospital wards, and resistant patterns, in which the number in groups is also less than five where fisher exact test was applied. In addition, independent T-test was used to examine the mean age of the subjects.
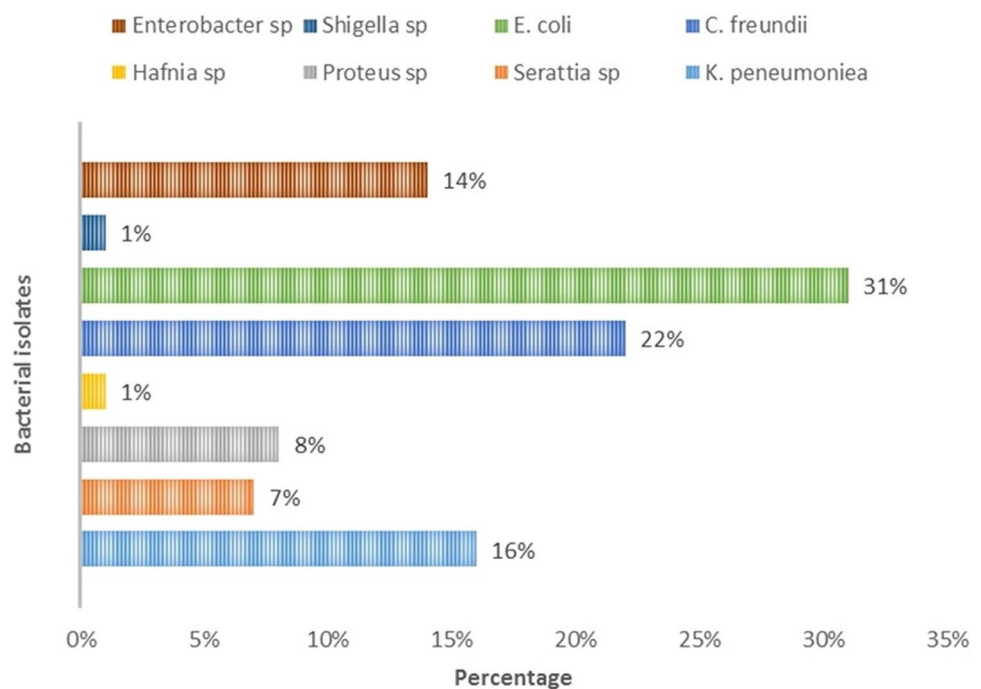
Results

Out of 2645 clinical samples, 853 (32.25%) from Bouali and 1792 (67.75%) from Zareh Hospital), 297 (11%) isolates were identified as Enterobacteriaceae, using biochemical methods as confirmed by molecular techniques. The mean age of the patients was 42.08 ± 25.08 years (minimum age 6 months and the highest age was 88 years) and 56% of the patients were male. There was no significant difference between males and females in terms of mean age ($p = 0.64$). The rate of Enterobacteriaceae isolation from Bouali and Zareh hospitals was 131 (4.96%) and 166 (6.28%), respectively. The most common isolates in Zareh and Bouali hospitals were *Citrobacter freundii* 63 (37.95%) and *Escherichia coli* 68 (51.9%), respectively. The total frequency showed that *E. coli* 93 (31%), *C. freundii* 65 (21.9%), *K. pneumoniae* 48 (16.2), *Enterobacter spp* 43 (14.5), *Proteus* 23 (7.7), S*erratia* 20 (6.7), *Hafnia* spp. 3 (1%) and *Shigella* 2 (0.7%) were respectively the most common isolates (Fig. 1). The highest bacterial isolation rates of clinical specimens were observed among patients under 10 years of age and over 70. Generally, the highest and lowest incidences of *E. coli* isolates were associated with ICUs 47 (50.5%) and NICU 8 (8.6%) wards,

respectively. Most *K. pneumoniae* are isolated from the ICU, at 34 (70.8%). Figure 2. All *C. freundii* isolates were related to burn and BICU (Figs. 2 and 3). The prevalence of isolated bacteria based on center and clinical sample type are shown in Fig. 4, 5a and 6a. In terms of clinical samples type, the majority of the isolates were related to urine 117 (39.4%) and wound 114 (38.4%). Frequency of clinical samples type as detail was as follow: wound (39.4%), urine (39%), sputum (11%), blood (7.7%), semen (1%), and stool (1%). (Fig. 4).

According to CDC instructions, the resistance pattern of confirmed strains ($n = 297$) to selected antibiotics was assessed and reported as resistant, intermediate and susceptible. Detailed data were shown in Tables 1, 2 and 3. The results of antibiotic susceptibility testing are listed in Table 2. There was no significant correlation between the resistance to the tested antibiotics and sample types and hospital wards where the samples collected. The best drug of choice for *E. coli* and *K. pneumoniae* isolates as the two predominant strains were amikacin (73–77%). Furthermore, most isolated *E. coli* have shown good sensitivity to imipenem (69.9%), meropenem (71%) and tobramycin (69.9%) and could be considered as the choice drug. Cefalexin did not yield acceptable results in the susceptibility tests, as it had the highest resistance rate among all isolated strains. The percentages of resistance to cephalexin were as follows: *C. freundii* (87.7%), *K. pneumoniae* (87.5%), and *E. coli* (69.9%). Antibiotic susceptibility results demonstrated that 195 isolates (65.7%) were MDR. The highest percentage of MDR strains were observed in Burn (79.6%) and BICU (76.1%) with ICU (55.6%), NICU (43.8%), and PICU (40%) being the next wards, respectively ($P < 0.001$).

Fig. 1 Prevalence of the isolated Enterobacteriacea spp., according to the types of isolates
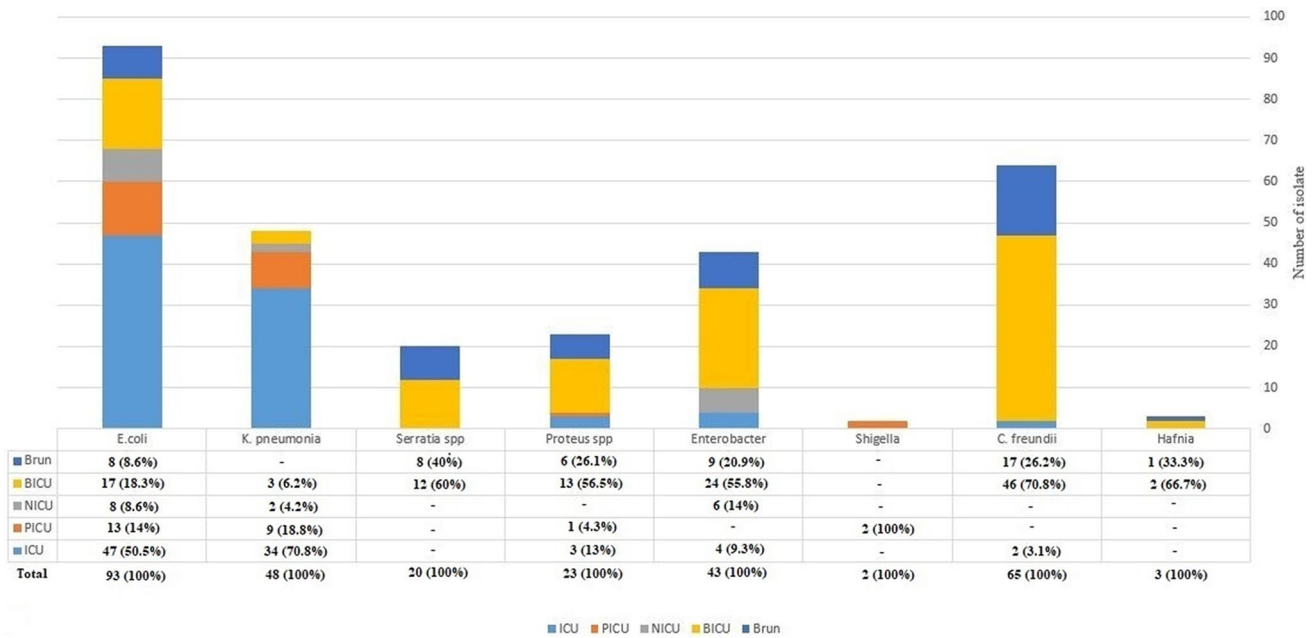
	E.coli	K. pneumonia	Serratia spp	Proteus spp	Enterobacter	Shigella	C. freundii	Hafnia
Brun	8 (8.6%)	-	8 (40%)	6 (26.1%)	9 (20.9%)	-	17 (26.2%)	1 (33.3%)
BICU	17 (18.3%)	3 (6.2%)	12 (60%)	13 (56.5%)	24 (55.8%)	-	46 (70.8%)	2 (66.7%)
NICU	8 (8.6%)	2 (4.2%)	-	-	6 (14%)	-	-	-
PICU	13 (14%)	9 (18.8%)	-	1 (4.3%)	-	2 (100%)	-	-
ICU	47 (50.5%)	34 (70.8%)	-	3 (13%)	4 (9.3%)	-	2 (3.1%)	-
Total	93 (100%)	48 (100%)	20 (100%)	23 (100%)	43 (100%)	2 (100%)	65 (100%)	3 (100%)

■ ICU ■ PICU ■ NICU ■ BICU ■ Brun

Fig. 2 Prevalence of the isolated Enterobacteriacea spp., according to the hospitals wards
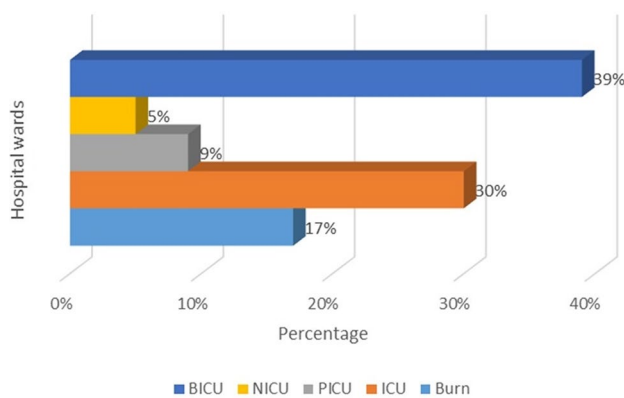

Fig. 3 Isolation rates of Bacterial isolates considering hospitals wards

The frequencies of isolates based on clinical specimens and hospital wards are shown in Fig. 4 and 5a for Bouali and Zareh hospital, respectively. MDR isolates were more often screened in Zareh 130 (78.31%) hospital. In addition, the MDR prevalence for *K. pneumonia*, Serratia spp., *C. freundii*, *Proteus* spp., Enterobacter spp., *E. coli*, and *Hafnia spp* were 100%, 90%, 87.3%, 84.2%, 69.7%, 56%, and 33.3%, respectively. The MDR isolation rates of Bouali and Zareh centers are detailed in Figs. 5b and 6b.

The relationship between sex, hospital wards, clinical specimens and bacterial infection were found to be statistically not significantly different ($p > 0.05$). There is no contingency at sex, hospital wards, and resistant patterns, in which the number in groups is also less than five where

fisher exact test was applied. The statistical analysis showed no significant differences ($p > 0.05$) in terms of the resistance of the isolates to the different antibiotics.

Discussion

Given the overuse of antibiotics in hospitals with exacerbation of antibiotic resistance, the appearance of new resistant strains, declining clinical outcomes, higher mortality rates, and excessive consumption of healthcare resources have been regarded as global health challenges [13, 14]. In this study, we tried to determine the spread of the MDR-*Enterobacteriaceae* following the prevalence assessment of the mentioned bacterial species in various clinical samples and hospitals wards. Our findings indicated that, out of 2645 clinical specimens, 297 (11.2%) were identified as Enterobacteriaceae spp. based on biochemical and molecular gold standard approaches [15]. *E. coli* was the most frequent (31.3%) isolate. Another study performed in Iran, agreed with our study in this research, Klebsiella spp., *P. aeruginosa* and *E. coli* were more prevalent than other bacteria[16] The cross-sectional studies in Uganda, Nepal, and Chad reported Enterobacteriaceae prevalence at 47%, 15%, and 63% respectively. Moreover, the proportion of isolated *E. coli* in the mentioned studies was 83.9% and 63.8% respectively [15, 17–19].

Because of the importance of Enterobacteriaceae spp., frequencies in HAIs and the mortality and morbidity rates of infections caused by this family of bacteria, much research
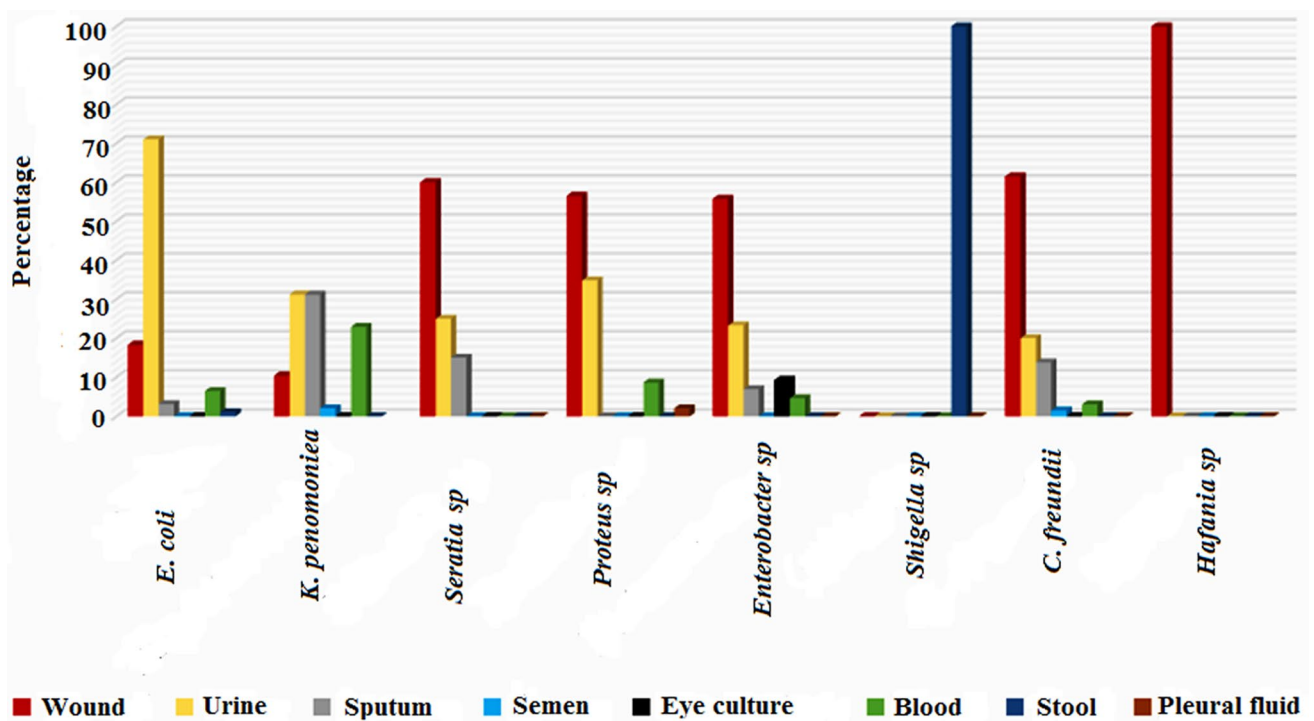
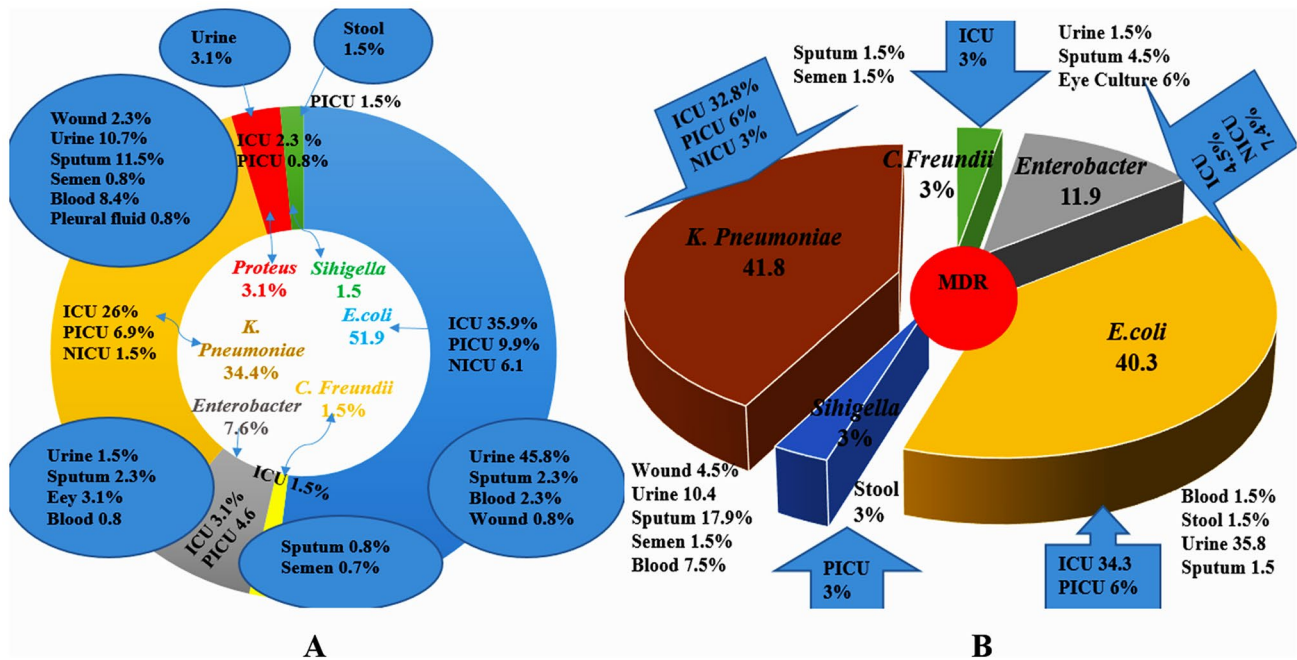Fig. 4 A cumulative data to Isolation rates based on the clinical specimence

Fig. 5 Prevalence of Isolated strains in Zareh hospital. (**a**) isolates percentage based on the clinical speciemence and hospital wards, (**b**) frequency of MDR isolates concidering Wards and clinical speciemens

has been conducted in recent years [17]. *E. coli* typically account for 40.8% of the bacteria isolated from uncomplicated UTI urines in Bouali center. In Zareh hospital, 50.9%

of urine specimen cultures were positive to the mentioned bacterium. The analysis of clinical isolates in this study suggests that clinically relevant MDR bacteria are prevalent in
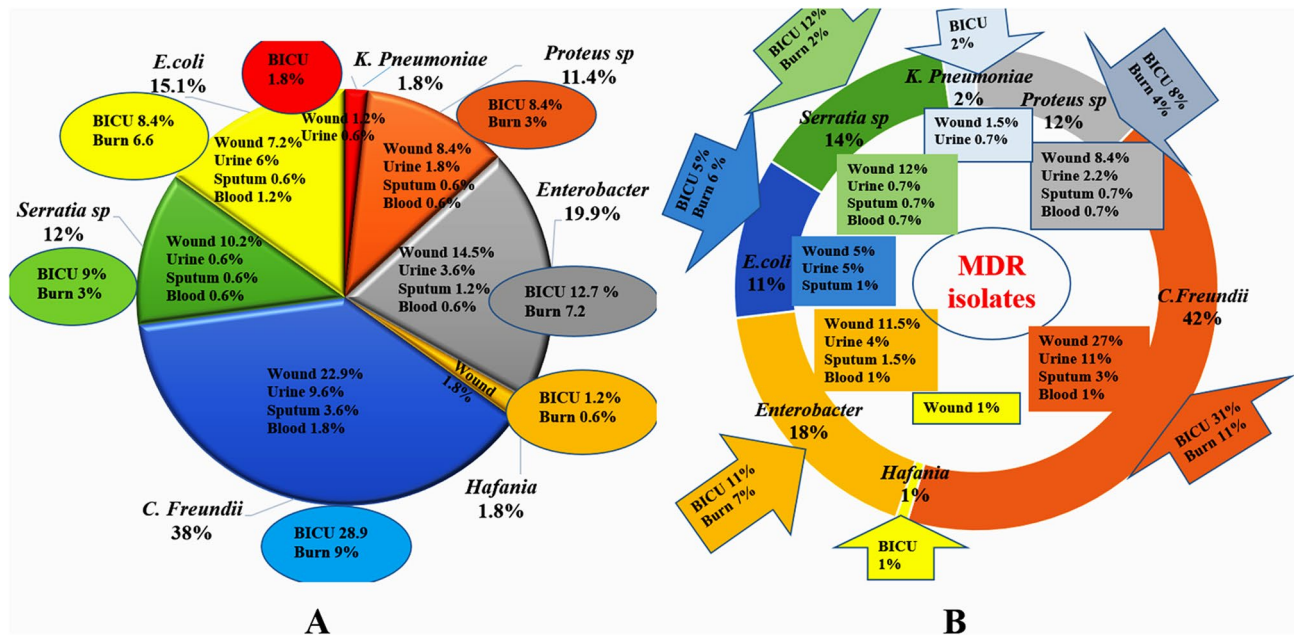
Fig. 6 Prevalence of Isolated strains in Bouali hospital. (**a**) isolates percentage based on the clinical speciemence and hospital wards, (**b**) frequency of MDR isolates concidering Wards and clinical speciemens

the studied patient population. Of special concern is the high percentage of *Enterobacteriaceae*, confirmed by both phenotypic and molecular assays.

In terms of sample size, similarities can be observed between the previously mentioned results and our findings. *K. pneumoniae* was the third most predominant isolate in our study (16.2%). Isolation rates of this bacterium were 21.2% and 40.1% in previously published research [15, 19]. Based on another cross-sectional study, out of 47% of Enterobacteriaceae spp. cases, 64.9% and 47.4% were isolated from urine and pus [20]. Moreover, frequencies of *E. coli* and *K. pneumoniae* isolates in the mentioned study were 53.9% and 28.7%, respectively [20].

After extensive use of antibiotics, drug-resistant strains have reported from different countries of the world [21]. Our study shows a high prevalence of nosocomial infections in our hospitals and in overall a high antibiotic resistance as MDR (65.7%) among the pathogens. In the present research, the most frequencies of MDR bacteria were *Klebsiella spp* and *Serratia spp*, respectively. Data showed that MDR isolates were more assessed in *K. pneumonia* (100%), *Serratia spp* (90%), *C. freundii* (87.3%), too. Considering the sample size our finding shown similarities to the mentioned studies in Iran and reconfirmed them.

Currently, non-response problems for Enterobacteriaceae include the development and the spread of resistance to many antibiotic classes, including broad-spectrum penicillin, third-generation cephalosporins, fluoroquinolones, aminoglycosides and carbapenems [22]. In treating patients,

the antibiotic resistance pattern of this bacterium has been widely studied in recent years. Harihana et al. (2015) indicated that the main part of *E. coli* (82.8%) and *K. pneumoniae* (93.1%) isolates were highly resistant to ampicillin. Moreover, estimated resistance rates of *E. coli* to ceftazidime, tetracycline, and ciprofloxacin are 34.5%, 72.41%, and 77.6%, respectively. The susceptibility patterns of *K. pneumoniae* isolates to piperacillin (50%), meropenem (41.7), and ceftazidime (41.4%) were assessed as well [15]. In the current research, the resistance percentages of *E. coli* and *K. pneumoniae* strains to tested antibiotics were as follows: ceftazidime (45.2%, and 68.8%), ciprofloxacin (23.5%, and 64.5%), piperacillin (72%, and 100%), ampicillin (71% and 87.5%), and meropenem (29% and 58%), which is similar to the findings of the research conducted earlier in India[15]. As shown in Tables 2, the cefalexin was respectively the top two least effective antibiotics in the present study, and Imipenem, meropenem (*E. coli*) and Amikacin (*E. coli* and *K. pneumoniae*) were the lowest rate of resistance. According to the antibiotic resistance pattern obtained in this study, cefalexin and ampicillin cannot treat infections related to the mentioned isolates. Also, depending on the antibiotic susceptibility results, imipenem, meropenem, tobramycin, and gentamycin could be considered either alone or in combination as drugs of choice for treating related infections.

Although significant carbapenem resistance rates of *E. coli* (29%) and *K. pneumoniae* (58.3%) are reported in this survey, amikacin and meropenem or imipenem could be used to prevent *E. coli*-associated disorders. Given the

Table 1 Prevalence of isolates according to the age and hospital

Age	Hospital	E. coli	k. pneumonia	Serratia spp	proteus spp	Enterobacter spp	Shigella spp	C. frundii	Hafania spp	Total
0–10	BO ALI	19 (48/7)	11(28.2)	–	1 (2.6)	6 (15.4)	2 (5.1)	–	–	39 (100)
	Zareh	2 (13.3)	–	1 (6.7)	1 (6.7)	2 (13.3)	0	7 (46.7)	2 (13.3)	15 (100)
11–20	BO ALI	2 (100)	–	–	–	–	–	–	–	2 (100)
	Zareh	3 (30)	–	1 (10)	2 (20)	3 (30)		1 (10)	–	10 (100)
21–30	Zareh	5 (16.7)	–	3 (10)	3 (10)	8 (26.7)	–	11 (36.7)	–	30
31–40	BO ALI	2 (100)	–	–	–	–	–	–	–	2 (100)
	Zareh	2 (6.2)	1 (3.1)	8 (25)	6 (18.8)	7 (21.9)	–	8 (25)	–	32 (100)
41–50	BO ALI	3 (50)	2 (33.3)	–	–	1 (16.7)	–	–	–	6 (100)
	Zareh	–	1 (4.5)	1 (4.5)	3 (13.6)	4 (18.2)	–	13 (59.1)	–	22 (100)
51–60	BO ALI	7 (36.8)	12 (63.2)	–	–	–	–	–	–	19 (100)
	Zareh	9 (37.5)	–	1 (4.2)	2 (8.3)	5 (20.8)	–	7 (29.2)	–	24 (100)
61–70	BO ALI	9 (60)	4 (26.7)	–	1 (6.7)	1 (6.7)	–	–	–	15 (100)
	Zareh	4 (15.4)	–	3 (11.5)	2 (7.7)	2 (7.7)	–	15 (57.7)	–	26 (100)
71–80	BO ALI	16 (69.6)	4 (17.4)	–	2 (8.7)	1 (4.3)	–	–	–	23 (100)
	Zareh	–	1 (20)	1 (20)	–	2 (20)	–	–	1 (20)	5 (100)
81–90	BO ALI	9 (47.4)	9 (47.4)	–	–	1 (5.3)	–	–	–	19 (100)
	Zareh	–	–	1 (50)	–	–	–	1 (50)	–	2 (100)
91–100	BO ALI	1 (16.7)	3 (50)	–	–	–	–	2 (33.3)	–	6 (100)
	total	93 (31.3)	48 (16.2)	20 (6.7)	23 (7.7)	43 (14.5)	2 (0.7)	65 (21.9)	3 (1)	297 (100)

Table 2 Antibiotics susceptibility patterns to isolated strains (Cumulative data to two centers)

		E. coli	*K. pneumonia*	Serratia spp	proteus spp	Enterobacter spp	Shigella spp	*C. frundii*	Hafania spp	*p value*
AN	S	68 (73.1)	37 (77.1)	6 (30)	9 (39.1)	24 (55.8)	2 (100)	29 (44.6)	1 (0.6)	0.412
	R	22 (23.7)	10 (20.8)	11 (55)	14 (60.9)	16 (37.2)	–	35 (53.8)	2 (1.8)	
	I	3 (3.2)	1 (2.1)	3 (15)	–	3 (7)	–	1 (1.5)	–	
GM	S	60 (64.5)	25 (52.1)	3 (15)	6 (26.1)	13 (30.2)	2 (100)	10 (15.4)	10	0.504
	R	31 (33.3)	21 (43.8)	17 (85)	17 (73.9)	30 (69.8)	–	54 (83.1)	–	
	I	2 (2.2)	2 (4.2)	–	–	–	–	1 (1.5)	–	
TOB	S	65 (69.9)	23 (47.9)	6 (30)	8 (34.8)	20 (46.5)	1 (50)	19 (29.2)	2 (66.7)	0.382
	R	27 (29)	25 (52.1)	14 (70)	15 (65.2)	22 (51.2)	1 (50)	45 (69.2)	1 (33.3)	
	I	1 (1.1)	–	–	–	1 (2.3)	–	1 (1.5)	–	
IPM	S	65 (69.9)	23 (47.9)	6 (30)	7 (30.4)	19 (44.2)	1 (50)	17 (26.2)	2 (66.7)	0.423
	R	27 (29)	25 (52.1)	14 (70)	16 (69.6)	23 (53.5)	1 (50)	48 (73.8)	1 (33.3)	
	I	1 (1.1)	–	–	–	1 (2.3)	–	–	–	
MEM	S	66 (71)	20 (41.7)	5 (25)	8 (34.8)	19 (44.2)	1 (50)	17 (26.2)	2 (66.7)	0.410
	R	27 (29)	28 (58.3)	15 (75)	15 (65.2)	22 (51.2)	1 (50)	48 (73.8)	1 (33.3)	
	I	–	–	–	–	–	–	–	–	
CN	S	28 (30.1)	6 (12.5)	1 (5)	5 (21.7)	7 (16.3)	–	8 (12.3)	1 (33.3)	0.501
	R	65 (69.9)	42 (87.5)	19 (95)	18 (78.3)	35 (81.4)	2 (100)	57 (87.7)	2 (66.7)	
	I	–	–	–	–	1 (100)	–	–	–	
CAZ	S	50 (53.8)	13 (27.1)	7 (35)	5 (21.7)	18 (41.9)	2 (100)	15 (23.1)	2 (66.7)	0.126
	R	42 (45.2)	33 (68.8)	13 (65)	17 (73.9)	24 (55.8)	–	50 (76.9)	1 (0.6)	
	I	1 (1.1)	2 (4.2)	–	1 (4.3)	1 (2.3)	–	–	–	
CP	S	49 (52.7)	23 (47.9)	5 (25)	7 (30.4)	16 (37.2)	1 (50)	13 (20)	1 (33.3)	0.114
	R	44 (47.3)	23 (47.9)	15 (75)	16 (69.6)	25 (58.1)	1 (50)	52 (80)	2 (66.7)	
	I	–	2 (4.2)	–	–	2 (4.7)	–	–	–	
NITRO	S	56 (60.2)	15 (31.2)	6 (30)	4 (17.4)	15 (34.9)	2 (100)	17 (26.2)	3 (100)	0.324
	R	30 (32.3)	30 (62.5)	14 (8.4)	19 (82.6)	26 (60.5)	–	48 (73.8)	–	
	I	7 (7.5)	3 (6.2)	–	–	2 (4.7)	–	–	–	
AM	S	25 (26.9)	6 (12.5)	3 (15)	1 (4.3)	5 (11.6)	–	13 (20)	1 (33.3)	0.487
	R	66 (71)	42 (87.5)	17 (85)	22 (95.7)	38 (88.4)	2 (100)	52 (80)	2 (66.7)	
	I	2 (2.2)	–	–	–	–	–	–	–	
CRO	S	53 (57)	16 (33.3)	8 (40)	10 (43.5)	17 (39.5)	–	23 (35.4)	1 (33.3)	0.412
	R	39 (23.5)	31 (64.6)	12 (60)	13 (56.5)	25 (58.1)	2 (100)	42 (64.6)	2 (66.7)	
	I	1 (1.1)	1 (33.3)	–	–	1 (2.3)	–	–	–	
NA	S	57 (61.3)	25 (52.1)	15 (75)	19 (82.6)	32 (74.4)	–	45 (69.2)	2 (66.7)	0.505
	R	36 (38.7)	23 (47.9)	5 (25)	4 (17.4)	11 (25.6)	2 (100)	20 (30.8)	1 (33.3)	
	I	–	–	–	–	–	–	–	–	
PIP	S	7 (28)	–	9 (45)	4 (21.1)	11 (33.3)	–	18 (28.6)	2 (66.7)	0.328
	R	18 (72)	3 (100)	11 (55)	15 (78.9)	21 (63.6)	–	45 (71.4)	1 (33.3)	
	I	–	–	–	–	–	–	–	–	
SXT	S	3 (12)	1 (33.3)	4 (20)	3 (15.8)	8 (24.2)	–	10 (15.9)	–	0.526
	R	22 (88)	2 (66.7)	15 (75)	16 (84.2)	24 (72.7)	–	52 (82.5)	3 (100)	
	I	–	–	1 (5)	–	1 (3)	–	1 (1.6)	–	

S = Sensitive, R = resistance, I = intermediate

AN = amikacin; CP = cefepime; CAZ = ceftazidime; CRO = Ciprofloxacin; GM = gentamycin; IPM = imipenem; MEM = meropenem; PIP = Piperacillin; ToB = tobramycin; AM = Ampicillin; SXT = Trimethoprim-sulfamethoxazole; FM = Nitrofurantoin; NA = Nalidixic; CN = Cephalexin. The statistical analysis (Chi-Square test) showed not significant differences ($p > 0.05$) in terms of isolates' resistance to all different antibiotics

Table 3 Frequency of antibiotic resistant bacterial isolates in each hospital separately

		E. coli	*K. pneumonia*	Serratia sp	Proteus sp	Enterobacter sp	Shigella sp	*C. freundii*	Hafnia
AN	B	4 (3.1)	8 (6.1)	–	–	1 (0.8)	–	–	–
	Z	18 (10.8)	2 (1.2)	11 (6.6)	14 (8.4)	15 (9)	–	35 (21.1)	2 (1.2)
GM	B	11 (8.4)	18 (13.7)	–	–	5 (3.8)	–	1 (0.8)	–
	Z	20 (12)	3 (1.8)	17 (10.2)	17 (10.2)	25 (15.1)	–	53 (31.9)	–
TOB	B	8 (6.1)	22 (16.8)	–	–	1 (0.8)	–	2 (1.5)	–
	Z	19 (11.4)	3 (1.8)	14 (8.4)	15 (9)	21 (12.7)	–	43 (25.9)	1 (0.6)
IPM	B	8 (6.1)	22 (16.8)	–	–	1 (0.8)	1 (0.8)	2 (1.5)	–
	Z	19 (11.4)	3 (1.8)	14 (8.4)	16 (9.6)	22 (13.3)	–	46 (27.7)	1 (0.6)
MEM	B	9 (6.9)	25 (19.1)	–	–	1 (0.8)	1 (0.8)	2 (1.5)	–
	Z	18 (10.8)	3 (1.8)	–	15 (9)	21 (12.7)	–	46 (27.7)	1 (0.6)
CN	B	42 (32.1)	39 (29.8)	–	1 (0.8)	9 (6.9)	2 (1.5)	2 (1.5)	–
	Z	23 (13.9)	3 (1.8)	19 (11.4)	17 (10.2)	26 (15.7)	–	55 (33.1)	2 (1.2)
CAZ	B	20 (15.3)	30 (22.9)	–	1 (0.8)	3 (2.3)	–	2 (1.5)	
	Z	22 (13.3)	3 (1.8)	13 (7.8)	16 (9.6)	21 (12.7)	–	48 (28.9)	1 (0.6)
CP	B	21 (16)	20 (15.3)	–	1 (0.8)	4 (3.1)	1 (0.8)	2 (1.5)	–
	Z	23 (13.9)	3 (1.8)	15 (9)	15 (9)	21 (12.7)	–	50 (30.1)	2 (1.2)
FM	B	10 (7.6)	27 (20.6)	–	3 (2.3)	4 (3.1)	–	–	–
	Z	20 (12)	3 (1.8)	14 (8.4)	16 (9.6)	22 (13.3)	–	48 (28.9)	–
AM	B	46 (35.1)	39 (29.8)	–	3 (2.3)	9 (6.9)	2 (1.5)	1 (0.8)	–
	Z	20 (12)	3 (1.8)	17 (10.2)	19 (11.4)	29 (17.5)	–	51 (30.7)	2 (1.2)
CRO	B	22 (16.8)	29 (22.1)	–	–	6 (4.6)	2 (1.5)	2 (1.5)	–
	Z	17 (10.2)	2 (1.2)	12 (7.2)	13 (7.8)	19 (11.4)	–	40 (24.1)	2 (1.2)
NA	B	30 (22.9)	22 (16.8)	–	1 (0.8)	5 (3.8)	2 (1.5)	–	–
	Z	6 (3.6)	1 (0.6)	5 (3)	3 (1.8)	6 (3.6)	–	20 (12)	1 (0.6)
PIP	B	–	–	–	–	–	–	–	–
	Z	18 (10.8)	3 (1.8)	11 (6.6)	15 (9)	21 (12.7)	–	45 (27.1)	1 (0.6)
SXT	B	–	–	–	–	–	–	–	–
	Z	22 (13.3)	2 (1.2)	15 (9)	16 (9.6)	24 (14.5)	–	52 (31.3)	3 (1.8)

AN = amikacin; CP = cefepime; CAZ = ceftazidime; CRO = Ciprofloxacin; GM = gentamycin; IPM = imipenem; MEM = meropenem; PIP = Piperacillin; Tob = tobramycin; AM = Ampicillin; SXT = Trimethoprim-sulfamethoxazole; FM = Nitrofurantoin; NA = Nalidixic; CN = Cephalexin; B: BOALI; Z: ZARE

overuse of antibiotics in Iran and the constant rise in antibiotic resistance, evaluation of resistant strains via antibiotic susceptibility testing seems to be critical to preventing the emergence of new resistant strains. Antibiotic resistance patterns not only differ in each country but also in each region due to genetic alterations and improper prescription of antibiotics [23]. In Iran, the rate of isolation of MDR for all species within the Enterobacteriaceae family is high. The rates range from 76.6% for *K. pneumoniae* isolated from the ICU patients,28 to 33% in other studies from non-ICU patients [24]. Many studies have been conducted to explore MDR and XDR Enterobacteriaceae spp. in recent years. While the high percentage of MDR isolates observed in various studies may be partly explained by the study design and over presentation of species with intrinsic resistance mechanisms (e.g. *Citrobacter* sp., *Enterobacter* sp.), even the usually less MDR species such as *E. coli* were found

to contain significant proportion of MDR strains [17]. In 2012 and 2017, some cross-sectional studies in Sudan and Iran reported percentages of MDR Enterobacteriaceae isolates spp. of 92.2% and 74%. Frequencies of MDR isolated *E. coli* (66.6%), *K. pneumoniae* (95.8%), and Enterobacter *spp.* (80%) strains were reported in a cross-sectional published in 2015 in Iran as well [5]. Based on the world health organization (WHO) reports in 2015, the prevalence of MDR Enterobacteriaceae spp. in African (≤4%), American (≤11%), Eastern Mediterranean (≤54%), European (≤68%), and western Pacific (≤8%) origins are reported. Italy and Liechtenstein were assigned as two more isolated MDR Enterobacteriaceae spp. countries of European origin with ≤50% and ≥50% frequency rates [13]. Our findings indicate that the prevalence rate of MDR isolates is 65.7%. Most of these cases were detected in BICU hospital wards. With respect to the sample size, our findings confirm previously

mentioned studies conducted in Iran [5, 25]. According to the previously published study, the most carbapenem-resistant Enterobacteriaceae (CRE) isolates were obtained from patients admitted in the hospital wards (42%) and ICU (26%) [26]. In the current study, isolation rates of *K. pneumoniae* (70.8%) and *E. coli* (50.9%) as two maim part of bacterial isolates in the ICU were screened. With respect to the sample size, the results were in line with our study. Moreover, main part of isolated bacteria was recovered from BICU (39%) and ICU (30%). These frequencies indicated that ICU infection controls procedure couldn't be effective to elimination of bacterial causative agents.

Conclusion

Clinically relevant MDR Enterobacteriaceae (higher numbers *K. pneumoniae* and *E. coli* were isolated compared to the other bacteria) are prevalent in the studied population, which may reflect a lack of a national antibiotic stewardship policy in the north of Iran, and over-the-counter access to antimicrobials. This situation, in turn, could lead to longer hospital stays, increased treatment costs, and higher mortality rates. These findings highlight an urgent need to develop new procedures for preventing the spread of genes that are responsible for XDR and MDR phenotypes, as well as for periodic evaluation of antibiotic susceptibility patterns of disease-related microorganisms.

Limitation

Responsible genes to antibiotic resistance and genetic relationship between the resistant strains are not determined and these are the limitation of this study.

Compliance with ethical standards

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