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Bloodstream Infections in the South of Iran: Microbiological Profile and Antibiotic-Resistance Patterns of Isolated Bacteria

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Abstract
Objectives: Bloodstream infection (BSI) causes significant morbidity and mortality in patients, especially in developing countries. Changes in the epidemiological pattern of microorganisms, as well as the growth of antibacterial resistance have become important health concerns in Iran. The aims of this study were to evaluate the spectrum of pathogens causing BSIs in hospitalized patients in Shiraz, Iran, and their corresponding antimicrobial resistance patterns.
Materials and Methods: In this retrospective study, 1585 positive blood samples were analyzed from March 2013 to March 2014. Samples from all hospitals within Shiraz were transferred to Professor Alborzi Clinical Microbiology Center. Then, the isolates were identified according to standard methods such as the analytical profile index system, and antibiotic susceptibility patterns were established consistent with the recommendations of the Clinical and Laboratory Standards Institute.
Results: Coagulase-negative staphylococci (39%), *Staphylococcus aureus* (15.3%), *Escherichia coli* (8.5%), *Pseudomonas* spp. (7.5%), *Enterococcus* spp. (7.3%), and *Acinetobacter* spp. (6.6%) were the most frequent bacteria isolated from blood cultures. Linezolid and vancomycin (VA) had the highest effectiveness against gram-positive bacteria and gram-negative bacteria had high sensitivity to polymyxin B and colistin. Totally, 56.2% of *Enterococcus* isolates were VA-resistant (VRE) and 55.2% (122) of *S. aureus* were methicillin-resistant (MRSA). Finally, 59.02% (72) of *E. coli* isolates, 33.3% (5) of *Serratia* spp., and 42.85% (33) of *Klebsiella* spp. were extended-spectrum β-lactamase (ESBL) positive.
Conclusions: Considering the results, the emergence of resistant strains such as MRSA, VRE, and ESBL is an alarming threat that would be a severe clinical issue with critical restrictions on antibiotic therapy.
Keywords: Bloodstream, Bacterial infections, Drug resistance, Microbial resistance

Introduction

Bloodstream infection (BSI) refers to the recovery of a microbial pathogen in the blood culture by virtue of infections rather than sample contamination (1). It is a life-threatening infection causing significant morbidity and mortality worldwide. The presence of living pathogenic bacteria in the blood of a patient usually represents a serious invasive bacterial infection requiring urgent antibiotic therapy. The BSI-associated mortality may range from 20% to 50% and depends on several factors including the pathogen and host. Many septic episodes in the BSI period are nosocomial and may be due to bacteria with increased antimicrobial resistance (2).

Gram-positive bacteria (e.g., *S. aureus* and enterococci) and gram-negative bacilli (e.g., *Enterobacteriaceae*, *Acinetobacter*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*) are the leading causes of nosocomial BSI. Antibiotic-resistant strains have emerged among gram-positive and gram-negative bacteria and

are being recognized increasingly (3,4). This marked increase in the incidence of bacterial infections due to antibiotic-resistant isolates in recent years is a great public health concern. It is presumed that infections caused by antibiotic-resistant strains result in greater morbidity, longer hospitalization, and mortality (5).

Despite progress in treatment and supportive care, BSI continues to be a major cause of morbidity and mortality in hospitalized patients. Accompanying the physiologic stress of infections is the increasingly added burden of multidrug resistance (MDR) that hinders the therapy of these infections and has adverse clinical and economic consequences. The ongoing emergence of resistant bacteria in the community and the hospital environment is a major threat to the public health system (6,7). The difference between nosocomial and community-acquired infections is becoming blurred as well since community-acquired organisms have become an important cause of hospital-acquired infections (8). Describing the

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importance of the problem with respect to these antimicrobial-resistant bacteria is challenging because the levels of antibiotic resistance vary for different types of healthcare facilities and various geographic regions. Infections with multidrug-resistant bacteria can lead to inadequate or delayed therapy and are associated with poor outcomes (6-8).

Rapid and reliable characterization of BSI, including the identification of bacteria with the species level and the determination of antibiotic resistance profiles, is vital for several reasons. Proper antibacterial drugs can be selected, and unnecessary treatment with less effective or ineffective drugs can be avoided accordingly. The prognosis of the patients can be improved, the acquisition of antibiotic resistance in bacteria may be decelerated, and finally, the charge on antibacterial and overall hospital costs can be diminished as well.

Therefore, this study mainly aimed at evaluating the spectrum of pathogens causing BSIs in hospitalized patients in Shiraz (Iran), as well as their antimicrobial resistance patterns, the characterization of extended-spectrum β-lactamase (ESBL), isolated methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant enterococcus (VRE) and the detection of MDR.

Materials and Methods
The study focused on pathogens responsible for BSIs and their antibiotic susceptibility patterns between 2013 and 2014 in Shiraz, Iran. The samples were collected from patients who referred to Nemazee hospital.

Isolation of Bacteria From Blood Samples
The required blood specimens were aseptically collected before the commencement of antibiotic treatment. The BACTEC Fluorescent series 9240 (Becton Dickinson, USA) instruments were used for rapid detection of bacteria in the blood samples. The specimens were collected in BACTEC standard culture vials for aerobic and facultative anaerobic bacteria. The bottles were loaded in the BACTEC machine within 30 minutes of sample collection. Whenever the machine gave an alert signal, the specific bottle was removed and gram-stain and subculture were done on microbiological media including blood agar, chocolate agar, and MacConkey agar.

Identification and Confirmation of the Isolated Bacteria
Gram-positive isolates were identified by traditional morphological and biochemical tests, including gram-stain, catalase, coagulase, DNase, bile esculin, hemolysis,

growth in 6.5% NaCl, optochin disk, and bacitracin disk (9). Gram-negative bacteria at the species level were identified based on gram-stain, oxidase test, catalase test, motility, triple sugar iron fermentation, DNase, colistin (CO) disk, colony morphology, and pigment production, and biochemical tests embedded in the API-20E and API-20NE biochemical kit system (bioMérieux SA, Marcy-1, Etoile, France) were used for final confirmation.

Brucella Isolation and Identification
At the physician's discretion, the blood for culture was drawn from febrile patients who referred to Nemazee hospital in Shiraz, the southwest of Iran. Sampling was performed when brucellosis was suspected based on epidemiological or clinical grounds. The required blood specimens were aseptically collected before the commencement of antibiotic treatment. BACTEC fluorescent series 9240 instruments (Becton Dickinson, USA) were applied for the rapid detection of bacteria from the blood samples, which were then inoculated into a BACTEC 9240 blood culture bottle. The bottles were sent to the clinical microbiology center and loaded into the BACTEC machine within 30 minutes of sample collection. The gram-stain of the broth was performed whenever detecting the positive bottle, and a portion of the fluid was subcultured on microbiological media, including blood and chocolate agars. The isolates were identified as *Brucella,* based on gram-staining, an atypical microscopic picture showing small gram-negative coccobacilli, positive oxidase, catalase, and rapid urease tests, negative fermentation of sugars, and colony morphology.

Antibiotic Susceptibility Testing
Susceptibility testing to antimicrobial agents (MAST Company, UK) was determined by disk-diffusion methods according to the clinical and laboratory standards institute (CLSI) recommendations (9). The results were evaluated based on the respective standards for antimicrobial susceptibility testing. The susceptibility of gram-positive isolates was tested for agents such as clindamycin (2 µg), erythromycin (15 µg), linezolid (LZD, 30 µg), penicillin G (10 µg), co-trimoxazole (SXT, 1.25/23.75 µg), rifampin (5 µg), oxacillin (OX, 1 µg), ciprofloxacin (CIP, 5 µg), chloramphenicol (C, 30 µg), and cephalothin (30 µg). The other agents were amikacin (AK, 30 µg), tetracycline (30 µg), vancomycin (VA,30 µg), quinupristin-dalfopristin (15 µg), gentamicin (GM, 10 µg), and gentamicin (HGM, 120 µg).

The susceptibility of gram-negative rods was tested for agents such as imipenem (IMP, 10 µg), meropenem (MEM, 10 µg), piperacillin-tazobactam (100/10 µg), CIP (5 µg), augmentin (AUG, 30 µg), piperacillin (100 µg), T (30 µg), cephalexin (CFX, 30 µg), ampicillin (AP, 10 µg), cefuroxime (30 µg), and C (30 µg). The other agents included levofloxacin (LEV, 5 µg), SXT (1.25/23.75 µg), amoxicillin (25 µg), cephalothin (30 µg), AK (30 µg), GM

(10 μg), tobramycin (10 μg), ceftriaxone (CRO, 30 μg), cefixime (CFM, 5 μg), cefotaxime (CTX, 30 μg), cefepime (CPM, 30 μg), ceftazidime (CAZ, 30 μg), aztreonam (ATM, 30 μg), ticarcillin (75 μg), and CO (10 μg).

For susceptibility testing of *Enterococcus* and streptococci, the bacterial culture was performed on Mueller-Hinton agar (MHA) supplemented with 5% sheep blood. Direct colony suspension, equivalent to a 0.5 McFarland standard, prepared by colonies from an overnight (18-20 hours) sheep blood agar plate was used for inoculation. Cultured plates were incubated in 5% CO_2 for 20-24 hours.

Brucella Susceptibility Testing

Susceptibility testing for different antibiotics was performed according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. Bacterial suspension, equal to 0.5 McFarland turbidity, was inoculated on MHA plates supplemented with 5% sheep blood, and discs were placed on media. The plates were incubated in ambient air at 35 °C, and the results were recorded after 48 hours.

Detection of MRSA
Cefoxitin (FOX) Disk and OX Disk

The isolates were tested with OX (5 μg) and FOX (30 μg) disks using MHA plates were inoculated with a suspension (equivalent to a 0.5 McFarland standard) of *S. aureus* clinical isolates. The plates were incubated at 35°C for 24 hours and inhibition zones were measured, and then the results were interpreted according to CLSI guidelines (10).

OX Agar Screen Plate

The OX agar screen plate, prepared in-house, well-performed in the detection of methicillin resistance in *S. aureus*. Ten microliters of the 10^6 CFU/mL bacterial inocula (final concentration =10^4 CFU/mL) was dropped onto MHA plates containing 4% NaCl and 6 μg/mL of OX. The isolate was considered to be OX resistant if any growth occurred within 48 hours of incubation at 33-35°C. *S. aureus* ATCC 25923 was tested with each batch of the medium as the standard strain (10).

Detection of Vancomycin Resistant S. aureus (VRSA) and VRE
Brain-Heart Infusion Agar Screen Plate

All *S. aureus* and enterococci isolates were examined for reduced VA susceptibility by the agar diffusion method. Then, 10 μL of a 0.5 MacFarland bacterial suspension (Final concentration =10^6 CFU/mL) was spotted on the BHI agar (Merck, Germany) containing 6 μg/mL VA, allowed to air-dry for approximately 5 minutes, and incubated at 35 °C. The plates were examined after 24 and 48 hours for any growth. For quality control, *Enterococcus faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were used as susceptible and resistant controls, respectively (10).

ESBL in *Enterobacteriaceae* Family Members
Combination Disc-Diffusion Method

All *Enterobacteriaceae* family members were screened for ESBL production according to CLSI guidelines using confirmatory disk-diffusion methods (11). CTX (30 μg) and CTX + clavulanic acid (30 + 10 μg), CAZ (30 μg) and CAZ + clavulanic acid (30 + 10 μg) discs (Mast, UK) were placed at a distance of 25 mm on an MHA plate, inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37 °C. A ≥ 5 mm increase in the diameter of the inhibition zone for the combination disc versus CAZ disc confirmed ESBL production. ESBL-producing strain *K. pneumoniae* ATCC 700603 and non-ESBL-producing strain *Escherichia coli* ATCC 25922 were used as positive and negative controls.

Results

A total of 1585 blood samples from patients with positive cultures were analyzed between 21 March 2013 and 20 March 2014. Based on the results, 55% of patients were males and the others were females. The mean age was 21.3±24.98 years ranging from 1 month to 91 years. Coagulase-negative staphylococci (CoNS, 39%), *S. aureus* (15.3%), *E. coli* (8.5%), *Pseudomonas* spp. (7.5%), *Enterococcus* spp. (7.3%), and *Acinetobacter* spp. (6.6%) were frequently isolated microorganisms from the bloodstream (Table 1). Totally, gram-positive bacteria (59.2%) were more frequent than gram-negative bacteria (40.8%).

The Susceptibility Patterns of Gram-Positive Bacteria

The antimicrobial susceptibility patterns of gram-positive bacteria are shown in Table 2. The highest rate of resistance in CoNS strains was found against penicillin (94.3%),

Table 1. Frequencies of Bacterial Strains Isolated From the Blood Cultures of Patients in Shiraz

Bacterial Species	Number of Isolates (%)
CoNS	562 (39)
Staphylococcus aureus	221 (15.3)
Escherichia coli	123 (8.5)
Pseudomonas	108 (7.5)
Enterococcus	106 (7.3)
Acinetobacter	95 (6.6)
Klebsiella	78 (5.4)
VGS	50 (3.4)
Enterobacter	32 (2.2)
Stentrophomonas	20 (1.3)
Serratia	17 (1.1)
Brucella	10 (0.6)
Proteus	6 (0.4)
Streptococcus pneumoniae	6 (0.4)
Salmonella	5 (0.3)
Total	1439 (100)

Note. CoNS: Coagulase negative staphylococci; VGS, Viridans group streptococci.

Table 2. Antibiotic Susceptibility Pattern of Gram-positive Bacteria Isolated From Blood Culture Between 2013 and 2014 at Nemazee Hospital, Shiraz (% Susceptible)

Antibiotic	Bacteria Name				
	S. aureus (n=221)	VGS (n=50)	CoNS (n=562)	*Enterococcus* (n=106)	*S. pneumoniae* (n=6)
Gentamicin 10	44.3	10	40.8	5.7	0
Gentamicin 120	-	-	-	31.1	-
Linezolid	100	100	100	96.2	100
Vancomycin	100	96	100	43.4	100
Rifampicin	57	94	68.3	4.7	100
Penicillin	1.4	40	1.8	16	-
Ampicillin	-	38	-	31.1	-
Oxacillin	31.2	28	18.9	0	33.3
Cotrimoxazole	60.6	14	22.6	12.3	-
Ciprofloxacin	38.9	26	37.4	4.7	66.7
Cefepime	33.5	86	32	4.7	100
Ceftriaxone	-	86	-	-	100
Cefixime	0.5	-	7.1	0	33.3
Cefoxitin	34.8	-	22.9	-	-
Azithromycin	-	24	-	1.9	33.3
Erythromycin	29.4	32	12.1	1.9	33.3
Clindamycin	36.6	72	21.9	5.7	33.3
Imipenem	-	98	-	35.8	100

Note. CoNS: Coagulase negative staphylococci; VGS, Viridans group streptococci.

followed by CFM (93.2%) and erythromycin (85.94%) while the lowest rate belonged to LZD and VA (0%).

Likewise, *S. aureus* isolates demonstrated the highest resistance against penicillin (95.5%), CFM (97.3%), and erythromycin (96.2%). Totally, 55.2% (122) of *S. aureus* isolates were MRSA. Among gram-positive bacteria, *Enterococcus* strains had a high rate of resistance to the majority of antibiotics, and the resistance rate was higher than 80% among antimicrobial agents. *Enterococcus* isolates were found to be the most susceptible to LZD (96.2%), and 56.2% of them were VRE. Only 33.3% of *S. pneumonia* were sensitive to OX by the disk-diffusion method predicting the sensitivity to penicillin. The minimum inhibitory concentration should be done for other strains.

The Susceptibility Patterns of Gram-Negative Bacteria
Escherichia coli was the most common gram-negative bacteria showing the highest resistance rate to AP (91%), AUG (85.3%), and CFM (81.3%) and was more susceptible to IMP, MEM, polymyxin B, CO, and AK (Table 3). Approximately all gram-negative bacteria had a high resistance rate to AP, AUG, and CFM. *Salmonella* spp. had the lowest frequency which was susceptible to all the tested antimicrobial agents. The results further revealed that 75% of Enterobacteriaceae isolates were resistant to three or more classes of antibiotics and were considered as MDR (12). Our data showed that 10% of *Brucella* isolates were resistant to tetracycline.

Extended-Spectrum β-Lactamase
Based on the obtained data, 59.02% (72) of *E. coli* isolates,

33.3% (5) of *Serratia* spp., 42.85% (33) of *Klebsiella* spp., and 41% (13) of *Enterobacter* spp. were ESBL positive.

Discussion
The present study was conducted to determine the most prevalent bacteria isolated from blood culture and their antimicrobial susceptibility patterns from March 2013 to March 2014 in Shiraz, Iran.

The most frequently isolated bacteria are listed in Table 1. Accordingly, the overall frequencies of gram-positive and gram-negative organisms were 66.5% and 33.5%, respectively, which are consistent with the results of some studies (12-16). Contrary to our results, gram-negative bacteria were predominant in most studies (17-19). Some factors including different setups in the blood culture system, the applied antibiotics in hospitals, geographic regions, and epidemiological differences between causative agents and seasonal variations can probably contribute to the variation of the isolated bacteria (20-22).

In the current study, CoNS (39%), *S. aureus* (15.3%), *E. coli* (8.5%), and *Pseudomonas* (7.5%) were the most frequent microorganisms that were isolated from the blood culture (Table 1). In a study in Tehran, CoNS, *S. aureus*, *Klebsiella* spp., and *E. coli* were the most prevalent isolates from the blood culture (23), which is in line with our findings. In a study conducted in Tabriz, *S. aureus* (4.18%) and *E. coli* (2.16%) were reported as the predominant microorganisms in BSIs (24). In the study by Arora and Devi, the incidence of *S. aureus* and CoNS was reported 27.37% and 20.1% (22) while in the study by Roy et al, this rate was 16.5% and 14% for CoNS and *S. aureus*, respectively (25). Similarly, CoNS was reported as the

Table 3. Antibiotic Susceptibility Pattern of Gram-negative Bacteria Isolated From Blood Culture Between 2013 and 2014 at Nemazee Hospital, Shiraz (% susceptible)

Antibiotics	Bacteria									
	Enterobacter (n=32)	*Klebsiella* (n=78)	*Pseudomonas* (n=108)	*Stenotrophomonas* (n=20)	*Acinetobacter* (n=95)	*E. coli* (n=123)	*Brucella* (n=10)	*Salmonella* (n=5)	*Serratia* (n=17)	*Proteus*
Gentamicin 10	56.2	66.2	40.7	50	24.2	55.3	100	100	70.6	100
Amikacin	75	64.1	45.4	60	72.4	90.3	100	100	70.6	100
Tobramycin	46.9	52.5	34.2	50	25.3	41.5	-	100	23.5	-
Aztreonam	59.4	51.3	22.2	0	-	43.9	30	100	64.7	100
Imipenem	93.7	84.6	50	0	17.9	100	70	100	100	100
Meropenem	75	78.2	41.6	0	10.5	95.1	-	100	94.1	100
Cephalexin	3.1	26.9	1.8	0	3.1	12.2	-	100	5.9	16.7
Cefepime	62.5	55.1	36.1	20	15.8	48.8	60	100	58.8	100
Ceftriaxone	50	46.1	13	0	10.5	30.9	80	80	47.1	100
Ceftazidime	62.5	51.3	50	50	14.7	43.1	-	100	64.7	100
Cefotaxime	59.4	46.1	11.1	0	9.5	34.1	-	80	53	-
Ceftizoxime	65.6	48.7	4.6	0	10.5	52	-	100	58.8	100
Polymyxin B	81.3	85.9	100	75	100	93.4	-	100	-	-
Colistin	71.9	83.3	100	75	100	88.6	-	100	-	-
Chloramphenicol	65.6	61.5	5.5	45	15.8	57.7	90	60	53	-
Tetracycline	65.6	43.6	19.4	5	17.9	18.7	90	80	5.9	16.7
Piperacillin-Tazobactam	75	71.8	56.5	45	23.1	87	50	100	88.2	100
Piperacillin	43.8	32.05	31.5	25	8.4	8.1	-	60	-	-
Augmentin	3.1	14.1	5.5	0	5.3	12.2	70	80	5.9	66.7
Ampicillin	0	9	2.8	0	5.3	4.9	30	60	0	66.7
Ciprofloxacin	81.2	62.8	57.4	90	25.3	37.4	100	100	76.5	100
Co-trimoxazole	68.8	39.7	30.5	75	23.1	18.7	10	100	82.4	66.7

most prevalent bacteria in other studies (25,26). However, CoNs is often overrated since it is a common bacterial contamination in blood cultures (27). Among gram-negative bacteria, *E. coli* (8.5%) and *Pseudomonas* (7.5%) had the highest frequency. Dagnew et al and Gohel et al reported *Klebsiella* spp. and *E. colis* as the predominant gram-negative bacteria (15,16). Additionally, *Enterobacter* spp., as an important nosocomial pathogen, was observed in 2.2% of BSI infections while in other studies by Arora et al and Roy et al, *Enterobacter* isolates were 14.19% and 22.9%%, respectively (21,24). Gohel et al reported that *Enterococcus* spp. with an incidence rate of 7.3% is among the most prevalent isolates, which have been reported to be 3.8% of isolates (16).

To decrease the mortality and morbidity rates of BSI emerged from gram-positive bacteria, the initiation of antimicrobial treatment at the earliest time is essential. Empirical therapy is usually prescribed based on common microorganisms and antibiotic susceptibility patterns in each center (28). Considering that the pathogen population and resistance to antimicrobial agents change over time, updating knowledge on local susceptibility patterns is critical for the selection of appropriate antibiotics. Antibiotic susceptibility patterns for gram-negative and gram-positive bacteria isolated from the blood are presented in Tables 2 and 3. According to the results, LZD and VA had the highest effectiveness against gram-positive bacteria. In our study, all staphylococcus isolates were VA sensitive, which is in agreement with the findings of other studies (13,16,29,30). However, Gohel et al and Kamga et al found that 21.6% and 32% of isolates were VA-resistant, respectively. VA, a glycopeptide, has been considered as the drug of choice for the treatment of MRSA infections (16,31,32). MRSA was detected in 55.2% of isolates in the current study, which was considerably higher than that of a comprehensive report in Iran (37.5%) (4). MRSA infections constitute approximately 40-60% of strains causing hospital-acquired infections. The results of another study in Iran indicated an increase in the incidence of MRSA (4). In our study, the prevalence of VRE was 56.2% while it was 7% in a study in Tehran (33). In addition, the corresponding rates were 40% and 6.3% in studies conducted by Gohel et al and Abebe et al, respectively (16,34). Based on the results of another study, 25% of *Staphylococcus* isolates were resistant to VA while all *Enterococci* were susceptible to VA (23).

Our findings showed that *Enterococcus* isolates had high resistance against all antibiotics and relative sensitivity to LZD (96.2%), IMP (35.8%), VA (43.4%), GM 120 (31.1%), and AP (31.1%), indicating a rising concern regarding the treatment of *Enterococcal* infection. VRSA was not detected in this study. Moreover, no VRSA strains were found in a study on the antibiotic susceptibility pattern of *S. aureus* (35).

According to our findings, polymyxin B and CO showed the highest effectiveness against gram-negative bacteria, and maximum resistance was observed to AP and AUG. Arora et al and Japoni et al reported an increasing incidence of AP resistance in isolated microorganisms (22,36). Furthermore, an increase in the prevalence of AP resistance was found in other studies (14, 26). Among the gram-negative bacteria, *Stenotrophomonas maltophilia* isolates revealed high resistance to most applied antibiotics in this study with 100% resistance to ATM, IMP, MEM, CFX, CRO, CTX, ceftizoxime, AUG, and AP. *E. coli* isolates exhibited high sensitivity (>90%) to AK, carbapenems (IMP and MEM), and polymyxin B, which is consistent with the results of Poorabbas et al (4).

Another interesting finding of our study was the more frequent occurrence of resistance to CPM (40%), IMP (30%), CRO (20%), and T (10%) among *Brucella* isolates. The occurrence of MRSA, VRE, and ESBL isolates found in our study is quite alarming. The potential risk for MDR emerging in nosocomial bacteria could have important clinical implications in infection treatment protocols. The prevalence of MDR and highly resistant strain traits in our study could also be regarded as an alarming situation. These antibiotic-resistant pathogenic bacteria can come from a variety of foods and other sources (35-38).

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