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REVIEW

The threat of carbapenem-resistant gram-negative bacteria in a Middle East region

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Abstract: Data on the status of carbapenem-resistant microorganisms in the Middle East countries are scarce. The aim of this review was to collect available data regarding resistance to carbapenems in a Middle East region. Available data regarding carbapenem-resistant isolates were considered for evaluation in this review. Biomedical electronic databases were systematically searched to find related articles. The key terms used were "carbapenem-resistant, resistant gram-negative bacilli, *Enterobacteriaceae*, fermenting and non-fermenting gram-negative bacilli, *Pseudomonas*, *Acinetobacter*, *Klebsiella* and Iran". After primary screening, 275 relevant articles were selected to be assessed thoroughly. Resistance rate to carbapenems was reported between 1% and 86% during years 2006–2018. Most of the carbapenem-resistant microorganisms were isolated from burn patients. Modified Hodge test was a commonly used phenotypic test. Only in few studies, genotypic assays were considered. Pattern of antibiotic use can affect emergence of resistant microorganisms. Rational use of drugs, and specifically, antibiotics is a challenging issue in developing countries. Mean number of drugs per prescription in these countries was higher than the World Health Organization standards. Overuse of antibiotics, especially injectable ones, and easy access to antibiotics without prescription is a warning alarm for future antibiotic resistance in developing countries. Establishing antimicrobial stewardship's programs is new in the hospitals. Unfortunately, rules and regulatory issues to restrict antibiotic access in community pharmacies and prescription by general physicians are limited.

Keywords: carbapenem, resistant, gram-negative bacteria, antibiotics

Video abstract

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Introduction

Carbapenem-resistant gram-negative bacteria are now a global concern around the world. Most of these strains are also resistant to other antimicrobial agents including aminoglycosides and fluoroquinolones.[1] Few therapeutic options without the desired efficacy are available to treat infections caused by carbapenem-resistant microorganisms.[2] This real threat necessitates applying reliable methods to determine prevalence of these isolates.

The methods to detect carbapenemase-producing microorganisms are divided into two classes: phenotypic and genotypic methods. Phenotypic methods are nonmolecular assays that detect structure of the carbapenemase enzyme (mostly active site) through a chemical or microbiological process.[3] The carbapenemase enzymes are structurally classified according to their active sites. Ambler class A (known as *Klebsiella*-producing carbapenemase [KPC]) has serine amino acid in its active site and can be inhibited by β-lactamase inhibitors. Ambler class B has zinc in its active site and is called metallo-β-lactamase (MBL), so it can react with EDTA and dipicolinic acid (DPA). The last

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class, Ambler class D, has serine in its active site, but it may or may not be inhibited by β-lactamase inhibitors.[4] According to these classes, various phenotypic methods are proposed to detect carbapenemase enzyme in gram-negative isolates.

Genotypic methods are targeted to detect resistance genes (mostly *bla* type genes) and polymerase chain reaction (PCR) is a common method. Other genotypic method is clonal typing.[5] Although detection of resistant-encoding genes is more accurate, however, these methods are usually expensive and not available anywhere.[6] Some other mechanisms of resistance to carbapenems including loss of porins, efflux pump mutation, and target site inactivation have been identified.[7] Figure 1 shows different genotypic and phenotypic methods for detection of resistance to carbapenems.

Data on the status of carbapenem-resistant microorganisms in the Middle East countries are scarce. The aims of this review were to collect available data regarding methods of detection and prevalence of resistance to carbapenems in a Middle East region.

Methods

Databases

Biomedical electronic databases (Scopus, Medline, Google Scholar, and Science Direct) were systematically searched to find related articles. The key terms used were "carbapenem-resistant, resistant gram-negative bacilli, *Enterobacteriaceae*, fermenting and non-fermenting gram-negative bacilli, *Pseudomonas*, *Acinetobacter*, *Klebsiella* and Iran". A total of 586 articles were found. After primary screening, 275 relevant articles were selected to be assessed thoroughly.

Study settings

Of the 275 articles, case reports, systematic reviews, meta-analysis, and articles in Persian language were excluded, and finally 97 English-language articles were included in the present review. These studies were published between 2006 and 2018 and were reported from different regions of Iran. Forty-eight studies were from Tehran and most of them were done at Motahari hospital (Level I Iranian burn hospital). The number of publications from other areas were as follows: ten in Isfahan, eight in northwest of Iran, five in south of Iran, four in Shiraz, three each in Hamadan, Arak and Mazandaran, two each in Shahrekord, Kermanshah, Mashhad, and Zanjan, and one each in Kerman, Khorramabad, and Qom. All the studies considered the standards and guidelines of the clinical and laboratory standards institute (CLSI) for the preparation and interpretation of the tests. Figure 2 illustrates data processing in this review.

Clinical and microbiological setting

All the studies included inpatients, with eight also considering outpatients. If the source of the samples was specific, they

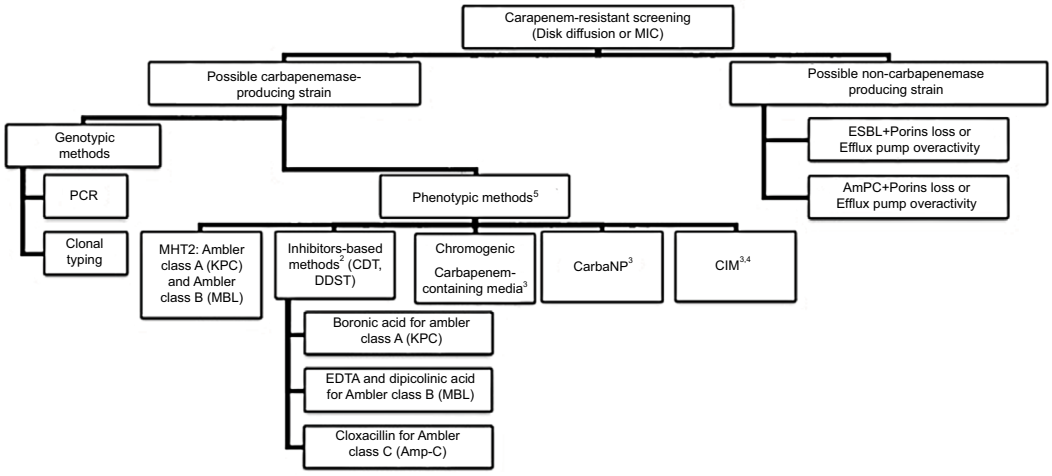

Figure 1 Screening methods for carbapenem-resistant microorganisms.

Notes: [1]MHT was the most used phenotypic method in Iranian studies. MHT originally detects KPC, but if zinc sulfate is added to MHT culture media, it can detect MBL. [2]It is based on carbapenemase inhibition by betalactamase inhibitors. CDT consists of two disks: a carbapenem and the other combination of a carbapenem and a betalactamase inhibitor. If the disk with inhibitor shows a bigger inhibition zone, the result is considered positive. DDST consists of carbapenem discs at a variable distance to inhibitor discs. The observation of synergy between disks is noted as a positive result. [3]These phenotypic methods can detect different Ambler classes. [4]CIM is a newer phenotypic method that was introduced in 2015 by van der Zwaluw. A carbapenem disk is inserted in culture media of suspected strain. Then it is transferred to another culture media with known control strain. If the suspected strain contains carbapenemase enzyme, the carbapenem disk has been degraded and the control strain in second culture will grow. [5]Some other phenotypic methods like spectrometric assays are also used and have higher sensitivity and specificity, but they are costly and time consuming.

Abbreviations: CDT, combination disk test; CIM, carbapenem inactivation method; DDST, double disk synergy test; ESBL, extended spectrum β-lactamase; KPC, *Klebsiella*-producing carbapenemase; MBL, metallo-β-lactamase; MHT, modified Hodge test; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction.

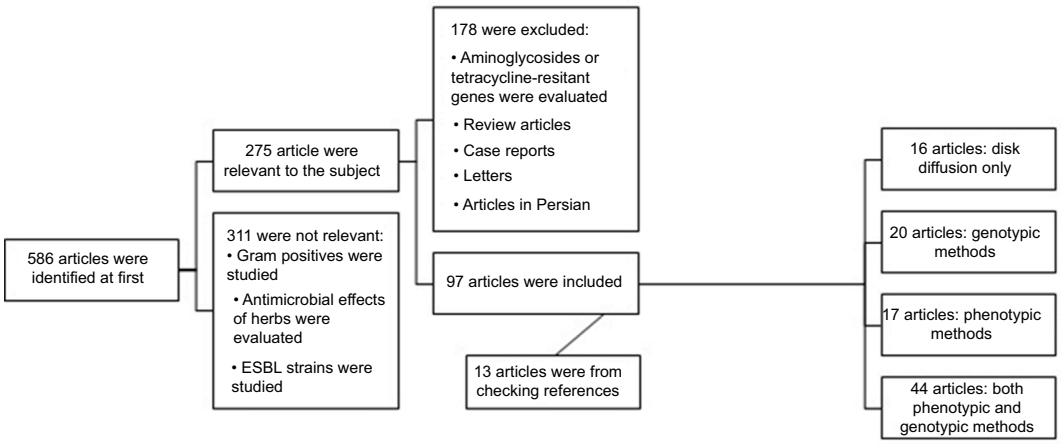

Figure 2 Consort flowchart of study.
Abbreviation: ESBL, extended spectrum β-lactamase.

are mentioned and if they were collected from different sites, they are named as "different sites".

All studies have been categorized into four distinct groups:

1. Studies that reported carbapenem-resistant microorganisms according to the disk diffusion method
2. Studies that considered phenotypic methods
3. Studies that used genotypic methods
4. Studies that applied both genotypic and phenotypic assays

Results

Studies that reported carbapenem-resistant microorganisms according to the disk diffusion method (early detection of resistance to a carbapenem)

In this category, detection of resistance to a carbapenem was described only by disk diffusion method. However, in some of them, minimum inhibitory concentration (MIC) values were determined. No phenotypic or genotypic assay was considered for carbapenemase detection. A total of 3,396 isolates were reported in this group (Table 1).

Screening of resistance to a carbapenem according to the disk diffusion method was common among the studies.[8–23] Although this method may be applicable for many antimicrobials, however, for carbapenems more confirmatory tests beyond the disk diffusion assay are needed. In this setting, MIC would help for detection of resistant isolates.

Five studies were multicenter,[8–12] but the sample sizes for three of them were small.[10,13,14] The trend of resistance to a carbapenem did not follow a specific pattern. However, in two studies,[12,15] 40% of the isolates were resistant to a carbapenem.

Studies that considered phenotypic methods

A total of 4,264 isolates were included in this category and *Acinetobacter* spp. and *K. pneumonia* were the common isolates. Modified Hodge test (MHT) was used as a common phenotypic test in most studies. MHT can detect Ambler class A (KPC), although it may be positive with the presence of other carbapenemase enzymes. Other applied tests were combination disk test (CDT) for detection of MBL or extended spectrum β-lactamase and the double disk synergy test for detection of MBL. In the study by Raategar Lari et al,[24] 92% of *A. baumannii* isolates were resistant to imipenem and only 24% of them were MBL positive in the CDT test, but in the next study,[25] 54% of isolates were resistant to imipenem and all of them were KPC producers according to the phenotypic tests.

The rates of resistance to aminoglycosides and fluoroquinolones were similar to those of carbapenems, although no special trend can be followed through different years. The results are provided in Table 2.[26–41]

Studies that included genotypic methods

A total of 2,195 strains were included in this group and except for three studies, all assessed *Acinetobacter* spp. mostly *A. baumannii*. PCR was performed in order to determine types of *bla* genes. In some studies, MIC values were correlated with the presence of *bla* genes. In the study by Taherikalani et al the MIC values of 256 mcg/mL were detected in strains with more than two bla_{OXA} genes.[42] In the study by Azizi et al, higher MIC value was associated with presence of bla-$_{\text{OXA-24/40 like}}$.[43] Bahador et al detected IS_{Aba} *genes*.[44] When these elements were placed upstream of OXA-type genes, they enhanced the expression of OXA-type genes. Presence of

Table 1 Screening of carbapenem-resistant isolates (disk diffusion method)

Author (year)	Setting	Population	Samples	Microorganism (No.)	MIC[a]	Resistance rate to FQ and AG	Results[b]	Weak points	Strength points
Mobaraki et al (2018)[15]	Hospitals in Tabriz	Inpatients and outpatients	Different sites	*Pseudomonas aeruginosa* (200)	–	TOB 57% GEN 62% CIP 62.%	• 46.5% of isolates were resistant to IPM	–	• Multicenter study
Ghanbari et al (2017)[16]	One hospital in Fuldshahr	General inpatients	Urine	Different strains (317)	–	[c]	• 9.5% of *Escherichia coli*, 11% of *Klebsiella* and 25% of *Proteus spp.* isolates were resistant to IPM	–	• Large sample size
Chahoofard et al (2017)[17]	One hospital in Bandar Abbas	General inpatients	Urine	Different strains (296)	–	[c]	• Resistance rate to IPM: *Enterobacter spp.* 7.1% *Citrobacter spp.* 0% *E. coli* 4.9% Klebsiella *pneumonia* 19.2% *P. aeruginosa* 21.4% *Acinetobacter baumannii* 53.2%	–	• Adequate sample size • Clinical status of patients including duration of hospital stay and comorbidities were evaluated
Ansari et al (2017)[18]	Two hospitals in Shahrekord	General inpatients	Urine, blood, sputum, wound	*A. baumannii* (30)	–	NAL 80%, OFX 86 % LVX 66 %	• About 55% of strains were resistant to IPM and DOR • Intermediate resistance rate was 10%–33.3%	• Small sample size • AGs were not tested	• Cloning smpA gen
Douraghi et al (2016)[8]	Four hospitals in Tehran	General inpatients and outpatients	Different sites	*A. baumannii* (400)	✓	–	• 97% were resistant to carbapenems • MIC values of all resistant isolates were above 32 mcg/mL	–	• Multicenter study • Large sample size
Ghasemian et al (2016)[9]	Four hospitals in Mazandaran	General ICU patients	Different sites	*Acinetobacter spp.* (50)	✓	CIP 96% AMK 100%	• Resistance to IPM and MEM according to E-test was 100%, and according to disk diffusion was 76% and 96%, respectively • Most common site for sampling was endotracheal tube	• Small sample size • MIC values did not report	• Multicenter study
Babamahmoodiet al (2015)[13]	Three hospitals in Mazandaran	ICU patients	Wound, respiratory secretions, urine, blood	Different strains (114)	–	[c]	• 14% of *P. aeruginosa*, 60% of *Acinetobacter spp.*, 33% of *E. coli*, 16% of *Enterobacter* and none of *K. pneumonia* isolates were resistant to IMP	• Small sample size for each strain	• Multicenter study • Demographic features and risk factors for hospital-acquired infection were described

(*Continued*)

Table 1 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (No.)	MICa	Resistance rate to FQ and AG	Resultsb	Weak points	Strength points
Mohajeri et al (2014)[14]	Three hospitals in Kermanshah	General inpatients	Blood, urine, sputum	*Acinetobacter spp.* (104)	–	Not defined	• 48% of isolates were resistant to carbapenems (imipenem and meropenem). 76% of carbapenem-resistant strains were isolated from sputum samples	• Few antibiotics were tested	• Multicenter study
Babakhani et al (2014)[19]	One hospital in Khorramabad	General inpatients (mostly ICU)	Different sites	*Klebsiella spp.* (80)	–	GEN 52 % AMK 7.5% CIP 82 % OFX 75% NAL 60%	• 67% and 1% of isolates were resistant to IMP and MEM, respectively		–
Kamalbeik et al (2013)[20]	One Poisoning center in Tehran	ICU patients	Different sites	*Acinetobacter spp.* (40)	–	CIP 97%	• Resistance rates to MEM and IPM were 100% and 62% (27.5% intermediate resistant to IPM)	• Small sample size	–
Hashemi et al (2013)[21]	Two hospitals in Hamadan	General inpatients and outpatients	Different sites	*Enterobacteriaceae spp.* (574)	–	c	• Resistance rate to IPM was 32% in inpatients and 4% in outpatients. Resistance rates to IPM in inpatients: *Enterobacter* 56%, *Serratia* 54%, *Klebsiella* 35%, *E. coli* 32%, and *Proteus* 11%	–	• Large sample size
Shakibaie et al (2012)[10]	One hospital in Kashan	ICU patients	Different sites	*Acinetobacter spp.* (15)	✓	CIP 60% GEN 60% AMK 26%	• 73% of isolates were resistant to IPM • MIC range for resistant strains was 128–240 and MIC of IPM for seven isolates was 240 mcg/mL	• Very small sample size	–
Rahbar et al (2010)[22]	One hospital in Tehran	General inpatients	Different sites	*P. aeruginosa* (109) *Acinetobacter spp.* (88) Stenotrophomonas *maltophilia* (48) *Burkholderia cepacia* (12)	–	c	• 1 %, 16%, and 98% of *A. baumannii*, *P. aeruginosa*, *and S. maltophilia* isolates were resistant to IPM, respectively	–	• Demographic and medical data of patients were provided

(*Continued*)

Table 1 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (No.)	MIC[a]	Resistance rate to FQ and AG	Results[b]	Weak points	Strength points
Soroush et al (2010)[23]	One pediatric hospital in Tehran	General pediatric patients	Different sites	*Acinetobacter spp.* (145)	–	In 2007: CIP 79% AMK 58% KAN 71% GEN 65% TOB 61%	• Resistance rate to IPM and MEM was analyzed in years 2006 and 2007 that was about 50%	• Carbapenem resistance was evaluated only in the last 2 years (2006–2007)	• Evaluated carbapenem resistance status in pediatrics • Change in the antibiotics resistance patterns was considered • Site of sampling with highest count of MDR strains in each year considered
Yousefi et al (2010)[11]	One hospital in Orumie	General inpatients and outpatients	Different sites	*P. aeruginosa* (160)	✓	–	• Approximately 40% of isolates were resistant to IPM, which means MIC for them was 31 mcg/mL • Presence of Class I integron and being in ICU and burn unit led to significantly higher IPM-resistant strains	• Just IPM was tested	–
Rahbar et al (2008)[12]	One hospital in Tehran	General inpatients and ICU patients	Not defined	Different strains (202)	✓	[c]	• 40% and 7% of *P. aeruginosa* and 38% and 27% of *A. baumannii* isolates were resistant to MEM and IMP, respectively • MIC range value for MEM was 0.5–32 mcg/mL • The majority isolates of *P. aeruginosa* and *A. baumannii* had an MIC >32 µg/mL for MEM	–	

Notes: [a]MIC values of carbapenems (and not other antimicrobial agents) were determined. [b]Resistant rates were reported according to the disk diffusion test. The unit for all reported MIC values is mcg/mL. [c]For complete information about the resistance to AG and FQ, refer to the original reference.

Abbreviations: AG, aminoglycoside; AMK, amikacin; CDT, combination disk test; CIP, ciprofloxacin; DD, disk diffusion; DDST, double disk synergy test; DOR, doripenem; ERT, ertapenem; ESBL, extended spectrum β-lactamase; FQ, fluoroquinolones; GEN, gentamicin; ICU, intensive care unit; IPM, imipenem; KAN, kanamycin; LVX, levofloxacin; MBL, metallo-β-lactamase; MEM, meropenem; MHT, modified Hodge test; MIC, minimum inhibitory concentration; NAL, nalidixic acid; NOR, norfloxacin; OFX, ofloxacin; TOB, tobramycin.

Table 2 Studies that considered phenotypic methods for detecting carbapenemase enzymes

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC[a]	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points
Ghotaslou et al (2018)[26]	Five hospitals in East and West Azerbaijan	General inpatients	Different sites	*Enterobacteriaceae* (307)	–	• DD • BA and DPA for MBL detection	[c]	• Resistance rate to carbapenem was observed only in *K. pneumonia* (57 isolates; 25%–45%) • Of these 57 isolates, phenotypic methods revealed that 15%, 7%, and 3% isolates of *Klebsiella* had MBL, KPC, and OXA-48 genes, respectively • Most of the carbapenemase strains were isolated from urine and blood	• Small sample size for each species	–
Saadatian Farivar et al (2018)[27]	Three hospitals in Tehran	Inpatients and outpatients	Different sites	*Klebsiella pneumonia* (81)	–	• DD • MHT for MBL detection	OFX 65%, CIP 68%, NOR 66%, GEN 66%, AMK 51%, TOB 56 %, KAN 79%,	• Resistance rate to IPM was 45% • 6 (7.4% of all) isolates were positive for MHT test	–	–
Moosavian et al (2014)[28]	Two hospitals in Ahvaz	General inpatients	Different sites	*Acinetobacter* spp. (100)	–	• DD • MHT	GEN 96% AMK 96% CIP 98%	• Resistance rates to IPM, MEM, and ERT were 95%, 96%, and 53%, respectively • 53% of all strains were MHT positive	–	–

(*Continued*)

Table 2 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC[a]	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points
Jonaidi Jafari et al (2014)[29]	One hospital in Tehran	General inpatients	Different sites	Different strains (350)	✓	• DD • CDT for ESBL detection	–	• MIC was determined by E-test • 95 strains were ESBLs • Resistance rates to IPM and MEM according to MIC, respectively, were: *Acinetobacter baumannii* 42%, 37% *Pseudomonas aeruginosa* 42%, 60% *K. pneumonia* 15%, 0%	• Small sample size for each microorganism	–
Ghadiri et al (2014)[30]	Three laboratories in Tehran	General outpatients	Urine	*Escherichia coli* (300)		• DD • CDT for ESBL detection • DDST for MBL detection	AMK 11% GEN 16%	• 67 isolates were ESBL producers and resistance to carbapenem in this group was about 10% • 21 isolates were MBL producers and resistance to carbapenems among them was almost 100% • 5 isolates were both MBL and ESBL positive	–	• Large sample size

(*Continued*)

Table 2 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC[a]	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points
Fazeli et al (2014)[31]	Teaching hospitals in Isfahan	General inpatients and ICU patients	Different sites	*K. pneumonia* (142)	–	• DD • CDT for ESBL	Resistance rate to GEN and AMK and CIP: 70%–80% LVX: 50%–60%	• Resistance rates to IPM, MEM, and ERT among all strains were 57%, 52%, and 47%, respectively • 101 strains were ESBL producers • Most of the ESBL producers were isolated from ICU and from urine samples	• It is not clear that the study was multicenter or not	–
Mirsalehian et al (2014)[32]	Motahari hospital	Burn patients	Probably burn wounds	*P. aeruginosa* (100)	✓	• DD • AmpC disk and CDT for AmpC production	–	• All IPM-resistant *Pseudomonas* were chosen and mean MIC for IPM was 64 mcg/mL • AmpC disk test and CDT were positive for 54 and 17 isolates, respectively showing a probable activation of one of the efflux pump and impermeability systems, or both	–	–

(*Continued*)

Table 2 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC[a]	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points
Moayednia et al (2014)[33]	One hospital in Isfahan	General inpatients and outpatients	Urine	*K. pneumonia* (484) and *E. coli* (1,080)		• DD • Double Disk for ESBL • MHT for KPC detection • CDT for MBL detection	Just reported for ESBL producer	• Resistance rate to carbapenem for *E. coli* was 1%–2% and for *Klebsiella* spp., it was 41%–46% • 390 isolates of *E. coli* and 238 *Klebsiella* were ESBL producers • From all of strains: *E. coli*: 2 MBL and 10 KPC producers *Klebsiella* spp.: 10 MBL and 186 KPC producers	• MIC was done by E-test, but cutoffs were not reported	• Large sample size
Erfani et al (2013)[34]	Three hospitals in Tehran	General inpatients	Different sites	*Acinetobacter sp.* (107)	–	• DD • CDT for MBL detection	CIP 95% LVX 93% GEN 51% TOB 64%	• Resistance rate to IPM and MEM was about 95% and 92% of these resistant isolates were MBL positive	–	–
Lari et al (2013)[24]	One hospital in Tehran (Motahhari)	Burn patients	Mostly burn wound	*Acinetobacter spp.* (69)	–	• SDDT for ESBL detection • CDT for MBL detection	GEN 64.7% AMK 95.7% KAN 97.1% TOB 2.9% CIP 97.1%	• Resistance rate to IPM was 92% • ESBL was positive in 10% of isolates • 24% of all baumannii isolates were MBL positive • The mortality rate in this study was 20%	–	• Mortality rate was reported • Detection of *Integron* genes

(*Continued*)

Table 2 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC[a]	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points
Rastegar Lari et al (2013)[25]	One hospital in Tehran (Motahari)	Burn patients	Burn wounds	*Klebsiella spp.* (35)	–	• DD • MHT for KPC production	AMK 71% GEN 72% TOB 86%	• Resistance rate to IPM was 54% and all were KPC producers • 7 out of 28 patients had two *Klebsiella* isolates with two different antibiotypes • Rate of mortality in patients infected with resistant strain was 33%	• FQ and colistin were not tested • MIC values were not reported • Small sample size	• Mortality rate was reported
Safari et al (2013)[35]	Three educational hospitals in Hamadan	ICU patients	Trachea, blood, urine, sputum, wounds	*A. baumannii* (100)	✓	• DD • E-test for MBL	Resistant: CIP 97% LVX 91% AMK 84% GEN 88%	• Resistance rate to carbapenem was 85%–94% but according to MIC, it was 87%–99% • 99% of all isolates were MBL producers		• Multicenter study • MIC was reported
Masaeli et al (2012)[36]	Two hospitals in Sanandaj	General inpatients	Different sites	Different strains (423)	–	• DD • DDST for MBL detection	GEN 70% AMK 80% CIP 37% NOR 42%	• The result of disk diffusion for carbapenem was not reported • 126 isolates (30% of all) were MBL positive • Risk factors like immunosuppression and use of antibiotics during the past 2 weeks were correlated with infection by MBL producers	• The resistance pattern of each group of microorganisms was not fully defined	• Correlation between being MBL and different clinical risk factors was assessed

(*Continued*)

Table 2 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC[a]	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points
Japoni-Nejad et al (2013)[37]	One hospital in Arak	General inpatients and environmental isolates	Different strains	*A. baumannii* (63)	✓	• MHT for KPC detection • E-test	Amikacin 80% Netilmicin 54%	• 84% were resistant to carbapenem • 47 isolates were MBL producers according to E-test and three of them were MHT positive • 60 strains had MIC more than 256	• Isolates from environment of hospital were also included	• Small sample size
Haji Hashemi et al (2012)[39]	Different hospitals in Tehran and Isfahan	General inpatients and outpatients	Different sites	*Klebsiella pneumoniae* (244)	–	• DD • MHT for KPC detection • CHROMagar for KPC detection	–	• MHT and CHROMagar were compared • From all, 30 and 36 isolates were KPC producers according to MHT and CHROMagar, respectively. The infection of burn wounds at the highest percentage: 27 cases (75 %)	• MIC was not reported	• Multicenter study

(*Continued*)

Table 2 *(Continued)*

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC[a]	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points
Azimi et al (2012)[40]	Two burn hospitals in Tehran	Infant and pediatric patients	Not defined (probably burn wounds)	*Klebsiella*, *Acinetobacter*, and *Pseudomonas* (64 strains from 20 patients)	–	• DD • MHT for KPC production • CDT for ESBL detection	Not defined	• 36 isolates were resistant to all tested antibiotic (not mentioned which antibiotics) except Colistin • Among these 36 isolates, 15 isolates were resistant to IPM • From these 15 isolates, 13 were KPC and 6 were ESBL producers	• Types of samples were not defined • The sample size is very small (7 *A. baumannii*, 16 *Enterobacteriaceae*, 12 *Pseudomonas*) • There is no precise data of resistance to each antibiotic	
Japoni et al (2006)[41]	One hospital in Shiraz	General inpatients	Different sites (mostly wound)	*P. aeruginosa* (70)	✓	• DD • DDST for ESBL • MHT for MBL detection	CIP 73% AMK 93% TOB 97%	• Resistance rate to carbapenems was 33% • MIC value for IPM was 2 and for MEM was 1 • MBL was not detected	• Small sample size	

Notes: [a]MIC values for carbapenems (but not other antimicrobial agents) were determined. [b]Resistance rates were according to the result of disk diffusion test. [c]For complete information about the resistance to AG and FQ, please refer to the original reference.

Abbreviations: AG, aminoglycoside; AMK, amikacin; BA, boronic acid; CDT, combination disk test; CIP, ciprofloxacin; DD, disk diffusion; DDST, double disk synergy test; DOR, doripenem; DPA, dipicolinic acid; ERT, ertapenem; ESBL, extended spectrum β-lactamase; FQ, fluoroquinolones; GEN, gentamicin; ICU, intensive care unit; IPM, imipenem; KAN, kanamycin; KPC, *Klebsiella*-producing carbapenemase; LVX, levofloxacin; MBL, metallo-β-lactamase; MEM, meropenem; MHT, modified Hodge test; MIC, minimum inhibitory concentration; NAL, nalidixic acid; NOR, norfloxacin; OFX, ofloxacin; TOB, tobramycin.

these elements in genetic contents of gram-negative isolates would lead to higher rate of resistance to carbapenems. Table 3 describes the details of each study in this category.[42,45–61]

Studies that considered both phenotypic and genotypic methods

A total of 6,759 isolates were identified in this category. In most studies more than one phenotypic method was applied. In the study by Hosseinzadeh et al, 29 strains were carbapenem resistant, in which 27 and 23 of them were MHT positive and carried bla_{NDM-1} gene, respectively.[62] However, in another study by Bina et al, although MHT tests were positive, no resistance gene was found.[63]

The detection of resistance genes was much lower for strains other than *A. baumannii*. In the study by Lari et al in 2015, only 9 strains of *P. aeruginosa* (out of 255) contained resistance genes,[64] but in the study by Bagheri Josheghani et al, at the same time, more than 90 strains (out of 124) of *A. baumannii* harbored resistance genes.[65] Data are summarized in Table 4.[66–105]

Discussion

Increase in the rate of resistance to carbapenem antibiotics among gram-negative isolates is a worldwide concern, especially in developing countries. The results of this review also showed this threat in the recent decade in a country of Middle East area, Iran. Although the prevalence of carbapenem-resistant gram-negative rods was reported according to the disk diffusion method in most studies, phenotypic and genotypic assays confirmed this pattern in some available surveys.

Resistance to carbapenem antibiotics in the same years in a country of neighborhood, Saudi Arabia, shows lower resistance rate in comparison with Iran.[106] In 2010, resistance to carbapenems was reported in 10%–66% of gram-negative isolates in Saudi Arabia, which was lower than that in Iran (86%).[11] The results were different in Europe. In the year 2006–2007, 4% and 85% of gram-negative isolates were carbapenem resistant in North and South of Europe, respectively. Although data were limited to year 2006, Iran had a better condition compared with the South of Europe, but worse than North of Europe.[107] The results of another study in year 2012 also confirmed this pattern in North and South of Europe.[108] In case of *P. aeruginosa*, MIC values that were reported for carbapenem-resistant strains were dramatically different from European countries. In year 2011, Castanheira et al detected a mean MIC value >2 mcg/mL for carbapenem-resistant *P. aeruginosa* in 14 European countries.[109] Yousefi et al in Iran reported an MIC >32 mcg/mL for most of carbapenem-resistant *P. aeruginosa* isolates. At these times, the CLSI and European Committee on Antimicrobial Susceptibility Testing cutoff values for carbapenem resistance were 2 and 8 mcg/mL, respectively.[11]

Incremental trend of resistance to carbapenem antibiotics was evident through 2006–2018. The least resistance rate was reported by Rahbar et al in year 2010 at Milad Hospital. In this year, 1.1% of *A. baumannii* isolates were resistant to imipenem.[22] This conclusion may be misleading, because 2 years earlier (in 2008), in this hospital, 27% and 40% of the same isolates were resistant to imipenem and meropenem, respectively.[12] Tarashi et al assessed the trend of antibiotic resistance in *A. baumannii* through years 2012–2015.[71] The results showed that resistance in *P. aeruginosa* increased from 83% in year 2012 to 96% in year 2015. Also 100% of *A. baumannii* were resistant to carbapenem among these years.

The resistance patterns for other microorganisms did not follow specific trends. For example, 60% of *P. aeruginosa* isolates were imipenem resistant in the year 2010,[101] but this rate was 25% in another hospital in the year 2016.[69] Outpatients-isolated *P. aeruginosa* strains showed less resistance rates (13% in Ahangarzadeh Rezaee et al[38] and 4.4% in Hashemi et al[21]). However, carbapenem-resistant rates were dramatically high in studies that included ICU samples.[9,20] In an earlier study,[9] 100% and 62% of *P. aeruginosa* isolates were resistant to imipenem and meropenem, respectively, and in a latter study,[20] about 100% of them were resistant to both carbapenems.

Most of the carbapenem-resistant microorganisms were isolated from burned patients, and many studies placed in this group were from Motahari Hospital. Motahari is a referral teaching burn hospital in center of Iran and most complicated burned patients are referred from all areas of the country. Increasing resistance rates can be observed through different years in burn patients; 50%–60% in year 2008 to 90% in year 2017.[42,46] Also, the presence of bla_{OXA} type genes has increased from half of the resistant strains to almost 80% of them. This can be a warning alarm regarding administration of antibiotics in burned patients.

Disk diffusion is the initial method for detection of resistant strains; however, it does not have enough accuracy for antimicrobial agents like carbapenem. Most evaluated studies considered disk diffusion method. Beside disk diffusion, MIC breakpoints can be used to confirm the results of disk diffusion (Figure 3).

Although MHT has been widely used as the preferred phenotypic method for detection of MBL, it is not recommended in latest version of CLSI, due to its low sensitiv-

Table 3 Studies that performed genotypic methods to detect genes encoding carbapenemase enzymes

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Found carbapenemase genes[a]
Sharikhani et al (2017)[45]	Four hospitals in Qom	General inpatients (mostly ICU)	Different sites	*Acinetobacter baumannii* (108)	–	• DD • PCR	CIP and AMK 93% GEN 81% LVX 91% TOB 47%	• 89% isolates were non-susceptible to IPM and MEM • Among carbapenem non-susceptible isolates, 82%, 55%, 22%, and 14% isolates had bla_{OXA-23}, bla_{OXA-58}, bla_{OXA-40}, and $bla_{OXA-143}$ genes, respectively	–	• First report of $bla_{OXA-143}$ in Iran • Multicenter study	bla_{OXA-51} bla_{OXA-23} bla_{OXA-40} bla_{OXA-58} $bla_{OXA-143}$
Mohammadi et al (2017)[46]	Two hospitals in Tehran	General inpatients	Burn wound and trachea	*A. baumannii* (103)	–	• DD • PCR	–	• About 90% of isolates were resistant to carbapenem • bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, bla_{TEM}, and bla_{PER} genes were detected in 90%, 38%, 1%, 60%, and 18.5% of all isolates, respectively • Significant relationship between the presence of bla_{OXA-24} and resistance to IPM (not MEM) • All strains had biofilm producing ability		• Ability of biofilm formation was assessed • Correlation between presence of genes and resistance to antibiotics was defined	bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, bla_{TEM}, bla_{PER}

(*Continued*)

Table 3 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Founded carbapenemase genes[a]
Bahador et al (2015)[44]	One hospital in Tehran	Burn patients	Not defined (probably burn wounds)	*A. baumanii* (62)	✓	• DD • PCR • Clonal typing	Not reported specifically	• According to MIC values, 61% were resistant to IPM • From all strains, 39 had bla_{OXA-23} (33 were susceptible to IPM), 9 had bla_{OXA-58}-genes • Existence of two genes (in 1.6%–19.3% of strains) was correlated with MIC more than 32 mcg/mL for IPM	• Resistance to each antibiotic was not reported specifically • Small sample size	• MIC values of IPM, tigecycline, and colistin were reported • ISAba gene was assessed	bla_{oxa-51} bla_{oxa-23} bla_{oxa-24} bla_{oxa-40}, $bla_{oxa-143}$
Kooti et al (2015)[47]	Four hospitals in Shiraz	General inpatients	Different sites	*A. baumannii* (200)	–	• DD • PCR	GEN 84.5% AMK 86.5% CIP and LVX 99.5%	• About 99% of isolates were resistant to IPM and MEM • 40% had bla_{OXA-23}, 7% had bla_{OXA-24}, 0.5% had bla_{OXA-58} • Two bla genes were detected in 4.5% of strains • The correlation between presence of genes and source of samples was not significant	–	Multicenter study	bla_{OXA-23} bla_{OXA_24} bla_{OXA-58}

(*Continued*)

Table 3 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Founded carbapenemase genes[a]
Azizi et al (2015)[43]	Two hospitals in Kerman	ICU patients	Different sites	*A. baumannii* (65 MDR strains from 266)	✓	• DD • PCR	CIP 100% AMK 78.5% TOB 93%	• MIC values for IPM and MEM for 76% of strains were >256 mcg/mL • bla_{oxa-23} was found in all isolates including sensitive and resistant isolates • Presence of $bla_{OXA\ 24/40}$ (29 strains) associated with higher MIC	–	–	bla_{oxa-51}, bla_{oxa-23}, $bla_{oxa-24/40}$
Mahdian et al (2015)[48]	One hospital in Tehran (Motahari)	Burn patients	Burn wounds, blood, urine	*A. baumanii* (37)	–	• DD • PCR • Clonal typing	CIP 100% GEN 94.6%	• 81% of strains were resistant to carbapenem o All resistant isolates had at least one bla_{OXA-23} and/or bla_{OXA-24} and about half of them had both genes	• Small sample size • Few antibiotics were tested	• MIC values for colistin and polymyxin B were reported • *ISAba* genes were assessed	bla_{OXA-23}, bla_{OXA-24}, bla_{TEM}, bla_{PER}

(*Continued*)

Table 3 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Founded carbapenemase genes[a]
Farsiani et al (2015)[49]	One hospital in Mashhad	General inpatients and ICU patients	Different sites	*A. baumannii* (36)	✓	• DD • PCR • Clonal typing	CIP 97%	• 97% of strains were resistant to carbapenems • MIC values of resistant isolates were >32 • bla_{OXA-51} and bla_{OXA-23} were detected in all isolates • From all isolates, bla_{OXA-24}, bla_{TEM}, bla_{ADC}, and bla_{VIM} genes were found in 23, 34, 22, and 23 strains, respectively • ISAba1 was detected in 97% of isolates of *A. baumannii*	• Small sample size	• *ISAba* genes and *tet* genes (related to efflux pump) were analyzed	bla_{OXA-51}, bla_{OXA-23} bla_{OXA-24}, bla_{TEM}, bla_{ADC} bla_{VIM} *adeB* *ISAba1* *tetA*, *tetB*
Nasrolahei et al (2014)[50]	Two hospitals in Tehran and Sari	ICU and burn patients	Trachea and burn wounds	*A. baumannii* (100)	–	• DD • PCR	STP 90% GEN 83% TOB 83% KAN 80%	• Resistance rate to IPM and MEM was about 70% • 67% of strain carried bla_{oxa-23} gene	• FQ are not tested	• Multicenter study	bla_{oxa-23}
Safari et al (2014)[51]	Three hospitals in Hamadan	ICU patients	Different sites	*Pseudomonas aeruginosa* (100)	✓	• DD • PCR	AMK 19% GEN 28% TOB 27% LVX 28% CIP 38%	• Resistance rate to carbapenems was about 20% • According to MIC values, 24% of isolates were carbapenem-resistant • Four isolates had bla_{IMP}		–	bla_{IMP}

(*Continued*)

Table 3 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Founded carbapenemase genes[a]
Bahador et al (2014)[52]	Two hospitals in Tehran	ICU patients	Different sites	*A. baumannii* (100)	✓	• DD • Clonal typing	[c]	• Resistance rates were compared among years 2006 and 2011, according to MIC cutoffs • In year 2006, resistance rate to IPM was 30% with MIC ≤4–64 mcg/mL. In year 2011 it was 48% and MIC values were ≤4 to ≥256 mcg/mL • Genotypes I and F were the most frequent genotypes in years 2006 and 2011, respectively		• Range of MIC values for each antibiotic was reported	–
Karmostaji et al (2013)[53]	Two hospitals in Tehran	General inpatients (mostly ICU)	Different sites	*Acinetobacter spp.* (131)	–	• DD • PCR	CIP 95% GEN 77% AMK 54%	• Resistance rate to IPM and MEM were 67% and 84%, respectively • 107, 17, and 1 of the resistant isolates carried bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA-58}, respectively. From these strains, seven strains had both bla_{OXA-23} and bla_{OXA-24}	–	–	bla_{oxa-51} bla_{oxa-23} bla_{oxa-24} bla_{oxa-58}

(*Continued*)

Table 3 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Founded carbapenemase genes[a]
Shoja et al (2013)[54]	Two hospitals in Ahvaz	ICU patients	Trachea	*A. baumannii* (206)	–	• DD • PCR	GEN 83%, TOB 78%, AMK 88%, CIP 96%,	• Resistance rate to IPM and MEM was 96% • bla_{OXA-23} and bla_{OXA-24} was found in 85% and 8% of all strains, respectively.	–	–	bla_{OXA-23} bla_{OXA-24}
Sohrabi et al (2012)[55]	One hospital in Tabriz	General inpatients	Different sites	*A. baumanii* (100)		• DD • PCR	CIP and GEN 86% LVX 84% AMK 81%	• 62% of isolates were resistant to carbapenems • Out of 62 resistant isolates, 55 carried bla_{OXA-23}, 1 carried bla_{OXA-40}, and 2 isolates had bla_{OXA-58} genes	–	–	bla_{OXA-23} bla_{OXA-58} bla_{OXA-40}
Sepehriseresht et al (2012)[56]	One hospital in Tehran (Motahari)	Burn patients	Burn wounds	*P. aeruginosa* (483)	–	• DD • PCR	–	• 56% of isolates were resistant to IPM, although 13% intermediate resistance should be considered • Among resistant isolates, 61 and 33 isolates had bla_{VIM} and bla_{IPM} genes, 23 strains had 2 resistant genes	• Origin of bla_{IPM} is not clear	–	bla_{VIM} bla_{IPM}

(*Continued*)

Table 3 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Founded carbapenemase genes[a]
Forozesh Fard et al (2012)[57]	One hospital in Isfahan	CF patients	Sputum	*P. aeruginosa* (11)	–	• DD • PCR	CIP 0% TOB 45.4%	• None of the isolates was resistant to IPM. None was positive for bla_{VIM} gene	• Very small sample size • Few antibiotics were tested	–	–
Peymani et al (2012)[58]	One hospital in Tabriz	General inpatients	Different sites	*A. baumannii* (68)	–	• PCR • Clonal typing	–	• All IPM-resistant isolates were chosen for study • 44% of isolates were from tracheal samples • All were positive for bla_{OXA-23}, and IS_{AbaI} was present upstream of all bla_{OXA-23} genes	–	–	bla_{OXA-23}
Asadollahi et al (2012)[59]	One teaching hospital in Tehran	ICU burn patients	Burn wounds	*A. baumannii* (23)	✓	• DD • PCR	CIP 100% GEN 30% AMK 47%	• Resistance rate to IPM was 48% • Range of MIC values for IPM was 0.004–32 mcg/mL • From all strains, bla_{PER} and bla_{TEM} genes were present in 52% and 43% of isolates, respectively. 21% and 17% of isolates had bla_{OXA-} and bla_{OXA24} genes, respectively	Small sample size	• MIC values were reported for each antibiotic • *CarO* and *tet* genes were assessed	bla_{TEM} bla_{PER} bla_{OXA-23} bla_{OXA-24} bla_{SHV}

(*Continued*)

Table 3 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Founded carbapenemase genes[a]
Taherikalani et al (2009)[42]	Six hospitals in Tehran	General impatients	Different sites	*A. baumannii* (80)	✓	• DD • PCR • Clonal typing	–	• 52% of isolates were carbapenem resistant • MIC_{50} range for carbapenem was 0.12–256 mcg/mL • Seven isolates that had MIC =256 mcg/mL had more than two bla_{OXA} genes • From all strains bla_{OXA-23} in 25%, bla_{OXA-58} in 21%, and bla_{OXA-24} in 15% genes were detected • Coexistence of multiple genes was observed	• Number of tested antibiotics were few	• Multicenter study	bla_{oxa-23} bla_{oxa-24} bla_{oxa-58}
Feizabadi et al (2008)[60]	One hospital in Tehran	General inpatients	Different sites	*Acinetobacter* spp. (*A. baumannii* =108 and other *Acinetobacter* =20, total =128)	✓	• DD • PCR	CIP 81% LVX 74% AMK 61% NTL 86% TOB 79% GEN 81%	• About 50% of strains were resistant to carbapenems • MIC values for carbapenem-resistant species were ≥64 to ≥256 mcg/mL • For details of isolated genes, refer to original article			bla_{oxa-23} bla_{oxa-24} bla_{oxa-51} bla_{oxa-58}

(*Continued*)

Table 3 (*Continued*)

Author (year)	Setting	Population	Samples	Microorganism (no.)	MIC	Method of carbapenemase detection	Resistance to FQ and AG	Results[b]	Weak points	Strength points	Founded carbapenemase genes[a]
Taherikalani et al (2008)[61]	Two hospitals in Tehran	Burn patients	Burn wounds	*A. baumannii* (38)	–	• DD • PCR	Resistance AMK 71% TOB 90% NTL 90% LVX 81% CIP 85%	• Resistance rate to carbapenem was about 60% • Half of the carbapenem-resistant strains had at least two bla_{OXA} *genes*	• Small sample size • MIC values were not determined		bla_{oxa-23} bla_{oxa-24} bla_{oxa-51} bla_{oxa-58}

Notes: [a]Results about bla_{OXA-51} were not provided because this gene has no correlation with occurrence of resistance, unless it has *ISAba* genes in its upstream. [b]Resistance rates were according to the results of disk diffusion test. [c]For complete information about the resistance to AG and FQ, please refer to the original reference.

Abbreviations: AG, aminoglycoside; AMK, amikacin; CIP, ciprofloxacin; DD, disk diffusion; FQ, fluoroquinolones; GEN, gentamicin; ICU, intensive care unit; IPM, imipenem; KAN, kanamycin; LVX, levofloxacin; MEM, meropenem; MIC, minimum inhibitory concentration; NAL, nalidixic acid; PCR, polymerase chain reaction; STP, streptomycin; TOB, tobramycin; NTL, netilmicin.

ity.[110] In CLSI 2018, CarbaNP method is recommended instead. Kuchibiro et al compared some phenotypic methods and MHT and showed an acceptable specificity (100%), but the sensitivity was very low (50%). Another test, modified carbapenems inactivation method, has acceptable sensitivity and specificity values (both above 99%) and does not require specific equipment. This method, beside CarbaNP, can be recommended for phenotypic detection of carbapenemase.[111]

Findings according to the phenotypic and genotypic methods were inconsistent. For example, in the study by Bina et al,[63] although all strains were MHT positive, none of them carry bla_{KPC} gene. This may be due to low sensitivity of MHT in detecting carbapenemase enzyme.

The genotypic assays were mostly applied on central regions of Iran. However, there are no data regarding carbapenemase-encoding genes in most provinces. This may be due to technical and economic restrictions in these areas (Figure 4).

The presence of some $bla_{OXA\text{-}type}$ genes were not necessarily associated to carbapenem-resistant. For example, $bla_{OXA\text{-}51}$ in *A. baumannii* did not contribute to carbapenem resistance at all. In the study by Azizi et al, it was shown that $bla_{OXA\text{-}23}$ may be found in both imipenem-sensitive and imipenem-resistant isolates.[43] Also, in the study by Bahador et al, $bla_{OXA\text{-}23}$ was found in both susceptible and nonsusceptible strains.[44] However, higher carbapenem resistance in isolates harboring $bla_{OXA\text{-}23}$ was reported (Shoja et al[54]). Table 5 shows the number of genes that were found in each city and related microorganisms.

Emergence of some genes including $bla_{NDM\text{-}1}$ is a serious global warning. This gene is highly transmissible and was first discovered in a Swedish patient who had traveled to Pakistan. Although almost all identified cases were originally from Pakistan and India, the first case of $bla_{NDM\text{-}1}$ that was reported in Iran in the year 2012, did not have a history of traveling to these countries. In two studies from Shiraz and Isfahan, 27 and 6 strains of *K. pneumonia* harbored bla_{NDM} in years 2015 and 2017, respectively.[62,78]

Genotypic methods to identify other mechanisms of resistance to carbapenems have recently been considered by researchers. *OmpK* and *carO* genes that regulate expression of mutated efflux pump were detected in Hashemi et al and Pajand et al studies, respectively.[83,93] In another study, efflux pump's activity and mutations in porins were investigated.[112] These results can expand the view about true mechanisms of resistance to carbapenems in developing countries.

Table 4 Details of studies that used both phenotypic and genotypic methods for detection of carbapenemase-producing strains

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Solgi et al (2017)[66]	Two hospitals (city was not defined)	General inpatients (mostly ICU)	Rectal swab	*Enterobacteriaceae* spp. (95)	✓	• MHT for MBL detection • Clonal typing	AMK 24% GEN 35% CIP 74%.	• 36 resistant strains were detected according to the phenotypic methods that among them carbapenem-resistance rates were between 88% and 100% • 47 isolates were MHT positive • 7 isolates carried bla_{NDM}, 23 carried bla_{OXA-48} • 17 isolates harbored two genes	• MIC values were determined, but not analyzed • Small sample size	• Risk factors for infection with resistant strains were investigated	bla_{NDM-1} bla_{NDM-7} bla_{OXA-48}
Khorvash et al (2017)[67]	One hospital in Isfahan	ICU patients	Different sites	*Pseudomonas aeruginosa* (48)		• MHT • PCR	AMK 79% CIP 85%	• Resistance rate to carbapenem was 97% • bla_{IMP} and bla_{VIM} were detected in 15 and 7 isolates (all were MHT positive) • In one isolate of *P. aeruginosa* with the source of urine, both bla_{IMP} and bla_{VIM} genes were detected • 52% of urine samples contained bla_{IMP}	–	• Ratio of MDR pathogens for each sample was defined	bla_{IMP} bla_{VIM}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Hosseinzadeh et al (2017)[62]	Two hospitals in Shiraz	General inpatients and ICU patients	Different sites	*Klebsiella pneumonia* (211)	✓	• MHT for MBL detection • DDST for MBL detection • PCR	AMK 78% CIP 85% GEN 89%	• 13% of isolates (29 ones) were resistant to IPM and MEM and had an MIC value of 1.5–32 mcg/mL (25 isolates had MIC ≥4 mcg/mL) • Among carbapenem-resistant isolates, DDST and MHT were positive for 27 strains • 2 and 27 isolates had bla_{OXA-48} and bla_{NDM}, respectively		• Large sample size	bla_{OXA-48}, bla_{NDM-1}
Akhi et al (2017)[68]	Seven hospitals in Tabriz and Orumie	General inpatients	Different sites	*P. aeruginosa* (245)	–	• DD • MHT • MCNP test • CIM • PCR	CIP 66% LVX 66% AMK 25%	• 49% (121) of isolates were carbapenem resistant • From 121 resistant strains, bla_{IMP} and bla_{VIM} genes were positive in 29 and 6 strains, respectively, and 40, 39, and 35 isolates showed positive results for MHT, MCNP, and CIM tests, respectively	• MIC was not tested	• Large sample size • Sensitivity and specificity of three phenotypic methods were estimated according to PCR • Multicenter study	bla_{VIM} bla_{IMP}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Falahat et al (2016)[69]	Three hospitals in Arak	General inpatients	Different sites	*P. aeruginosa* (108)	–	• MHT and CDT for KPC detection • PCR	CIP 20% AMK 21% GEN 23%	• Resistance rate to carbapenem was about 25% • 38 and 26 of all isolates were MHT and CDT positive respectively • 13 isolates had bla_{KPC} gene	–	• Multicenter study	bla_{KPC}
Mohammadzadeh et al (2016)[70]	Two hospitals in Tehran	General inpatients	Not defined (probably different sites)	Different strains (864)	–	• CDT, MHT, and DDST for MBL detection • PCR	Not defined	• 7% of isolates were IPM resistant • 35, 24, and 3 isolates were *Acinetobacter spp.*, *Pseudomonas spp.*, and *Enterobacteriaceae*, respectively • Carbapenem resistance was confirmed phenotypically (17% and 9%) and genotypically (15% and 9%) among *Acinetobacter* and *Pseudomonas*, respectively	–	• Large sample size	bla_{VIM}, bla_{IMP}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Tarashi et al (2016)[71]	One hospital in Tehran	Burn patients	Burn wounds	*Acinetobacter baumannii* (189) *P. aeruginosa* (309)	–	• CDT for MBL • PCR	In 2015 for *P. aeruginosa*: AMK and GEN 95% CIP 97% For *A. baumannii*: AMK and CIP 100% GEN 95%	• Carbapenem resistance rate of *P. aeruginosa* was 83% in 2012 and 96% in 2015 • Carbapenem resistance rate of *A. baumannii* in all years was 100% • Among 278 resistant *P. aeruginosa* isolates, 178 strains were MBL producers • Of the 187 resistant *A. baumannii*, 85 isolates were MBL producers • bla_{IMP-1} and bla_{VIM-1} genes were isolated in 30 and 52 of resistant strains of *P. aeruginosa*, respectively • bla_{IMP-1} and bla_{VIM-1} genes were detected in 10 and 34 of resistant *A. baumannii*, respectively	–	• Large sample size • Providing trend of resistance through different years	bla_{OXA-51} bla_{VIM} bla_{IMP}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Maspi et al (2016)[72]	One hospital in Tehran	General inpatients and ICU patients	Different sites (mostly BAL and wounds)	*A. baumannii* (86)	–	• CDT for MBL detection • PCR	TOB 75%	• Resistance rates to MEM and IMP were 90% and 73%, respectively • 44 isolates were MBL producers • 2, 13, 2, 4, and 2 isolates carried bla_{VIM}, bla_{IMP}, bla_{SPM}, bla_{GIM}, and bla_{SIM} genes, respectively	• MIC was not reported	• First report of bla_{SIM}, bla_{GIM}, and bla_{SPM}. genes	bla_{VIM} bla_{IMP} bla_{SPM} bla_{GIM} bla_{SIM}
Moghadam et al (2016)[73]	Two hospitals in Shiraz	General inpatients and ICU patients	Different sites	*A. baumannii* (98)	✓	• E-test for MBL detection • PCR	AMK 69% GEN 75% CIP and LVX 100%	• Resistance to carbapenem was about 95% and according to MIC, it was 98% • Except two, all resistant isolates according to MIC had breakpoints >16 mcg/mL • Out of resistant isolates, 43 strains were MBL producers • Out of MBL producer strains, 14 had bla_{VIM} and 23 had bla_{IMP} genes	–	• Results of general and ICU patients were discussed separately	bla_{VIM} bla_{IMP}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Pakbaten Toupkanlou et al (2015)[74]	One hospital in Tehran	Burn patients	Not defined	*P. aeruginosa* (50)		• CDT for ESBL detection • PCR	CIP 98%, AMK, GEN, and TOB 96%	• All resistant strains to IPM were chosen for study • 23 and 17 strains were positive in two different phenotypic methods for ESBL detection • 7, 18, 18, and 18 strains had bla_{PER}, bla_{OXA-10}, bla_{TEM}, and bla_{SHV} genes, respectively • 10 (20%) isolates carried bla-TEM, blaOXA-10, and blaSHV genes, simultaneously	• Small sample size • Origins of samples were not defined	–	bla_{OXA-10} bla_{SHV} bla_{TEM} bla_{PER}
Bina et al (2015)[63]	Three hospitals in Tehran	General inpatients	Different sites	*K. pneumonia* (270)	–	• MHT test for KPC detection • PCR	GEN 41.3%	Resistance rate to carbapenem was 14% (41 strains) • –14.5% (33 out of 41) strains were MHT positive and all 41 strains did not carry bla-$_{KPC}$ gene	• Few antibiotics were tested	• Large sample size • Multicenter study	–

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Eftekhar and Naseh (2015)[75]	Two hospitals in Tehran	Thirty nonburn and 25 burn patients	Different sites	*K. pneumonia* (30 nonburn and 25 burn, total 55)	–	• CDT for ESBL detection • DDST for MBL screening • MHT for MBL detection • PCR	Burn and nonburn patients respectively: CIP 84%, 60% AMK 52%, 33.3% GEN 84%, 56.7%	Resistance rates to carbapenem were 20% in burn and 0% in nonburn patients • ESBL production was positive in 83% of nonburn and 72% of burn isolates • None was an MBL producer and positive for KPC genes • MHT was positive in four strains • Carbapenem-resistant isolates (n=5) from burn wounds were resistant to all tested antibiotics	• Small sample size	• Comparison of resistance pattern in burn and nonburn patients	–
Lari et al (2015)[64]	One hospital in Tehran	Burn patients	Burn wounds	*P. aeruginosa* (255)	–	• CDT for ESBL production • DDST for MBL production • PCR	Were tested but not reported specifically	• All IPM-resistant strains were chosen for study • 63% were positive in CDT • None were positive for DDST • 5 and 4 strains had bla_{VIM} and bla_{IMP} genes, respectively	Resistance to other antibiotics was not defined clearly	• Large sample size	bla_{VIM} bla_{IMP}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Bagheri Josheghani et al (2015)[65]	One hospital in Kashan	General inpatients	Different sites	*A. baumannii* (124)	–	• CDT for ESBL detection • DDST for MBL detection • PCR	AMK 78% GEN 83% LVX and CIP 99%	Resistance rates to carbapenems was about 90% • 5% were ESBL positive and 54% isolates were MBL • 79% , 25%, and 3% carried bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA-58} genes, respectively	–	–	bla_{OXA-51} bla_{OXA-23} bla_{OXA-24} $bla_{OXA-58.}$
Azimi et al (2016)[76]	One hospital in Tehran	Burn patients	Burn wounds	Different strains (161)	–	• MHT and DDST for MBL detection • CDT for KPC detection • PCR	–	All IPM-resistant strains were included • 85, 51, and 112 of them were MHT, boronic acid, and dipicolinic positive, respectively	• Exact number of resistance genes was not reported	• The sensitivity and specificity of each phenotypic test were reported	bla_{VIM} bla_{IMP} bla_{OXA-23} bla_{OXA-48}
Azimi et al |(2015)[77]	One hospital in Tehran	Burn patients	Burn wounds	*A. baumannii* (65)	–	• CDT for ESBL detection • PCR	80% of strains were resistant to all AG	All IPM-resistant strains were selected for study • 1 strain was CDT positive • 83%, 12%, and 9% of all strains harbored bla_{OXA-23}, bla_{VIM}, and bla_{KPC} genes, respectively.	–	–	bla_{OXA-23} bla_{OXA-51} bla_{VIM}, *bla* KPC

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Fazeli et al (2015)[78]	One hospital in Isfahan	General inpatients	Different sites	*K. pneumonia* (112)	✓	• MHT and E-test for MBL detection • PCR	CIP 63.4% AMK 51% GEN 64%	42% of strains (49 isolates) were resistant to IPM and MEM • 32 out of 49 resistant isolates were positive for MHT • 5 isolates were positive in E-test and 6 strains harbored NDM-1 • MIC values for these six strains was >256 mcg/mL			bla_{NDM}
Khoshvaght et al (2014)[79]	Four hospitals in Zanjan	General pediatrics	Stool	*Escherichia coli* (230)	–	• DDST for ESBL and MBL detection • PCR	CIP 37% AMK 21% GEN 29%	IPM resistance rate was 2.1% • Out of the 36 Enteroaggregative *E. coli* isolates, 19 (52%) were ESBL positive and none were MBL positive • 15 and 12 isolates had bla_{TEM} and bla_{CTX-M} genes	• MIC was not tested	• Large sample size • Comparing data of healthy children with those with diarrhea	bla_{TEM} *and* bla_{CTX-M}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Noori et al (2014)[80]	Two hospitals in Tehran	General inpatients	Different sites	*Acinetobacter* spp. (108)	✓	• DD • CDT for MBL	CIP 92% AMK 80% GEN 40%	• 91% (99 isolates) were resistant to IPM and MEM • 86 isolates were MBL producers • Out of these 86 MBL producers, three isolates harbored *bla* $_{IMP}$ • MIC50 values for MEM and IPM were 32 and 128 mcg/mL, respectively	–	–	*bla*$_{IMP}$
Vali et al (2014)[81]	Two pediatric hospitals in Tehran	Cystic fibrosis inpatients	Sputum	Different gram negative (52)	✓	• CDT for ESBL detection • DDST for MBL detection • PCR	GEN 24.5% CIP 18%	• Resistance rate to MEM was 14% (eight strains) that MHT was positive in three of them • The EDTA disk synergy was also positive in four isolates • *bla*$_{VIM}$ and *blaIMP* were detected in five and two strains of *Achromobacter xylosoxidans*	–	• Data provided from cystic fibrosis patients	*bla*$_{CTX-M}$ *bla*$_{IMP}$ *bla*$_{VIM}$

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Azimi et al (2014)[82]	One hospital in Tehran	Burn patients	Burn wounds	*K. pneumonia* (28)	✓	• MHT • CarbaNP test • BA • PCR	GEN 90% AMK 79%	All isolates were resistant to IPM and MEM • 9 and 11 strains had an MIC >64 mcg/mL for IPM and MEM, respectively • All phenotypic tests except BA were positive • 27 isolates had bla_{OXA-48} gene and one had bla_{VIM-4}	• Small sample size • Few antibiotics were tested	• CarbaNP test was done	bla_{OXA-48} bla_{VIM-4}
Hashemi et al (2014)[83]	Two hospitals in Tehran	General inpatients and infants	Different sites	*K. pneumonia* (83)	✓	• CDT • AmpC screening • MHT • PCR	Resistance and intermediate: CIP 39.7%, 4.8% GEN 61.5%, 3.6% AMK 80.7%, 4.8%	• Resistance rates to IPM, MEM, and DOR were between 68% and 74% • MIC range for IPM and MEM was 0.25–256 mcg/mL • 48 (57%) isolates were positive for ESBL • 5 isolates were MHT positive • 23 isolates were AmpC positive • 30 strains had *OmpK35* genes	–	• Detection of porin-related genes	bla_{oxa-48} $bla_{CTX-M-15}$

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Japoni-Nejad et al (2014)[84]	One hospital in Arak	ICU patients	Surgical wounds, urine, blood, respiratory secretions	*K. pneumonia* (100)	–	• MHT • E-test for MBL detection • DDST • CDT for KPC detection • PCR	[d]	• Resistance rate to carbapenems in Amp C producers and carbapenemase producers was 5% and 66%, respectively • bla_{VIM} and bla_{GES} were detected in ten and two isolates • One isolate (1%) contained both bla_{VIM} and bla_{AmpC} genes	–	–	$bla_{VIM}bla_{GES}$
Farajzadeh Sheikh et al (2014)[85]	Hospitals in Ahvaz	General inpatients	Different sites	*P. aeruginosa* (223)	✓	• CDT and E-test for MBL detection • PCR	GEN 66% AMK 55% TOB 67% CIP 66%	58%, 31%, 13%, and 74% of isolates were resistant to IMP, MEM, DOR and ERT, respectively. Thirty isolates were resistant to all carbapenems • All imipenem-resistant isolates were MBL producers, except one • blaIMP and blaVIM genes were detected in 26 and 1 of the all strains, respectively • 13%–74% of resistant isolates had an MIC >16 mcg/mL for different carbapenems		• Large sample size	bla_{VIM} bla_{IMP}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Aghamiri et al (2014)[86]	Several hospitals in Tehran	General inpatients	Different sites	*P. aeruginosa* (212)	✓	• DD • DDST for MBL detection • PCR		47% of isolates were resistant to IPM and all had an MIC >16 mcg/mL • 70 isolates were MBL positive • 20 and 70 strains had bla_{IMP} and bla_{VIM} genes, respectively			bla_{IMP} bla_{VIM}
Nobari et al (2014)[87]	Eight hospitals in Tehran	General inpatients	Different sites	*K. pneumonia* (180)		• DD • MHT for MBL detection • PCR		Resistance rate to carbapenems was between 7% and 23% (66% of urine isolates were resistant to carbapenems) • 25 isolates were MHT positive • bla_{CTX-M}, bla_{SHV}, bla_{TEM}, and bla_{PER} genes were detected in 69%, 59.5%, 35.7%, and 16.6% of carbapenem-resistant isolates respectively • bla_{NDM-1}, bla_{VIM-1}, and bla_{KPC} were detected in 3, 5, and 1 isolates			bla_{CTX-M} bla_{SHV}, bla_{TEM} bla_{PER} bla_{NDM-1} bla_{VIM-1} bla_{KPC}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Hakemi Vala et al (2014)[88]	One hospital in Tehran	Burn patients	Burn wounds	*P. aeruginosa* (47) and *Acinetobacter spp.* (28)	✓	• CDT for MBL and ESBL detection • PCR	*P. aeruginosa* GEN 72% CIP 69% *A. baumannii* GEN 96% CIP 100%	• 100% of *A. baumannii* and about 75% of *P. aeruginosa* isolates were resistant to carbapenems • MIC range of IPM for *P. aeruginosa* was 2–128 and for *A. baumannii* was 4–128 mcg/mL • 17% of the *P. aeruginosa* isolates and 16% of the *A. baumannii* isolates were MBL producers • Mortality rate of MBL producer was 20%	–	• Mortality rate was reported	bla_{SPM}, bla_{IMP}, $bla_{CTX\text{-}M\text{-}15}$
Tavajjohi et al (2013)[89]	One hospital in Kashan	General inpatients and environmental isolates	Different strains and wet environment	*P. aeruginosa* (100)	–	• DDST for ESBL detection • PCR	GEN 50% CIP 12%	• 8% of isolates were ESBL producers that bla_{GES} was detected in all ESBL producers and 50% (4 isolates) of them were resistance to IPM	–	• Small sample size (only eight strains were evaluated for carbapenem resistance and genes)	bla_{GES}

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Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Azimi et al (2013)[90]	One hospital in Tehran (Motahari)	Burn patients	Burn wounds	*K. pneumonia* (44)		• DD • MHT for KPC detection • BA	–	• 64% of isolates were resistant to carbapenem • MHT was positive in all of them, but none showed synergism between MEM and boronic acid • All were negative for bla_{KPC}	• Other antibiotics were not evaluated • Small sample size	–	
Noori et al (2013)[91]	Two hospitals in Mashhad	General inpatients	Wounds and respiratory secretions	*Acinetobacter spp.* (70)		• DDST for MBL production • PCR	CIP 100% GEN 88%	• All isolates were resistant to IPM and MEM • 50 out of 70 strains were MBL producers, but only 3 strains had bla_{VIM}			bla_{VIM}
Azimi et al(2013)[92]	One hospital in Tehran	Burn patients	Not defined (probably burn wounds)	*A. baumannii* (93) and Acinetobacter *lwoffii* (1)	✓	• CDT for MBL detection • PCR	–	85% of isolates were resistant to IPM that MIC range for them was 16–128 mcg/mL • 34% of all isolates were MBL positive (31 strains) • 25 out of MBL producers had bla_{OXA-23} • None was positive in DDST			Bla_{oxa-23}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Pajand et al (2013)[93]	Two hospitals in Tehran	Inpatients and burn patients	Burn wounds and other sites	*A. baumannii* (43 burn and 32 nonburn patients, total=75)	✓	• DDST for MBL detection • PCR	Nonburn and burn patients: CIP 87%, 97% AMK 56%, 95% TOB 47%, 84%	Resistance rate to carbapenem in burn and nonburn patients was 98% and 68%, respectively • 66% of nonburn and 70% of burn isolates had bla_{OXA-23} • blaOXA-40-like gene was detected in 12% and 74% of non-burn and burn isolates, respectively • MIC values for all carbapenems were reported • All isolates were positive for AmpC and *ISAba* genes and 71 of them were positive for *carO* gene	• Results of DDST were not reported	• Detection of OMP gene (carO) for evaluation of carbapenem resistance	Bla_{OXA-23} bla_{OXA-40} *AmpC, ISAba, carO*
Azami et al (2013)[94]	Three hospitals in Tehran	General inpatients	Not defined	*P. aeruginosa* (130)	✓	• CDT for MBL detection • PCR	GEN 63% CIP 59%	• According to MIC, 53% of isolates were resistant to IPM and MIC values were ≥16 mcg/mL for IPM • 56% had class I integrons • bla_{VIM} was found in 10 strains	• Few antibiotics were tested • Source of sample was not clear	• Assessment of integrin genes (genes for mobility of betalactamase) • Multicenter study	bla_{VIM}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Mohajeri et al (2013)[95]	Three hospitals in Kermanshah	General inpatients	Sputum, blood, urine	*Acinetobacter spp.* (104)	–	• E-test for MBL detection • DDST • PCR	CIP 69%, GAT 43% LVX 62% TOB 39% GEN 68% AMK 53%	Resistance rate to carbapenems was 75%–80% • 84 of isolates were MBL producers • The bla_{OXA-23} and bla_{OXA-24} were found among 77% and 19% of the isolates, respectively • Correlation between rate of resistance to carbapenem and presence of bla_{OXA-23}-like gene was statistically significant	• MIC was not reported	• Multicenter study	bla_{OXA-23} bla_{OXA-24}
Doosti et al (2013)[96]	One hospital in Zanjan	General inpatients	Different sites	*P. aeruginosa* (70)	✓	• DD • DDST for MBL detection • PCR	CIP 40% GEN 55%	63% of isolates were resistant to IPM that 41 of them had an MIC >4 mcg/mL • DDST was positive for all of them • 36 isolates were MBL producers • From 41of isolates with high MIC breakpoints, 10 isolates had bla_{IMP} and 23 had bla_{VIM}	–	–	bla_{VIM} bla_{IMP}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Shahcheraghi et al (2013)[97]	Five hospitals in Tehran	General inpatients	Mostly urine and feces	Different strains (360)	–	• MHT • MBL E-test • PCR	CIP 61.3% AMK 14.4% KAN 38.6%	• 6%, 3%, and 1% were resistant to MEM, ERT, and IPM, respectively • MHT was positive in 11 resistant isolates	• MIC was not reported	• Multicenter study • Large sample size • Recognition of new *K. pneumonia* containing bla NDM	bla_{TEM} bla_{SHV} bla_{CTX-M} bla_{NDM} bla_{PER}
Kalantar et al (2012)[98]	One hospital in Sanandaj	General inpatients	Different sites	*P. aeruginosa* (100)	✓	• DD • DDST for MBL detection • PCR		• 22 isolates were MBL positive and among them 8 isolates were resistant to IPM • 8 isolates had bla_{VIM}			bla_{VIM}
Shahcheraghi et al (2011)[99]	Seven hospitals in Tehran	General inpatients	Different sites	*Acinetobacter spp.* (203)	✓	• DD • CDT for MBL detection • PCR	Of the 100 strains that were IPM resistant: CIP 84% AMK 84%	49% and 100% of isolates were resistant to IPM and MEM, respectively • All isolates had an MIC ≥8% and 47% had an MIC =64 mcg/mL • From IPM-resistant isolates, 9% were MBL producers • The bla_{SPM}, bla_{GES}, bla_{OXA-51}, and bla_{OXA-23} genes were detected by PCR among 6, 2, 94, and 84 isolates of *A. baumannii*, respectively		• Multicenter study • Large sample size • First report of bla_{SPM} and bla_{GES}	bla_{OXA-23} bla_{SPM} bla_{GES}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Peymani et al (2011)[100]	One hospital in Tabriz	General inpatients	Different sites	*A. baumannii* (100)	–	• E-test for MBL detection • PCR	AMK 83% GEN 91% CIP 90% LVX 86%	About 55% of isolates were resistant to carbapenem • 31 of carbapenem-resistant isolates were MBL producers and 28 of them were positive for bla genes		–	bla_{VIM} bla_{IMP}
Saderi et al (2010)[101]	One hospital in Tehran	General inpatients	Burn wounds	*P. aeruginosa* (100)	–	• CDT for MBL detection • PCR for MBL genes	GEN 86% AMK 73% CIP 55%	• 69% of isolates were resistant to IPM • 65 isolates were CDT positive • 13 resistant isolates contained bla_{VIM}	• MIC was not determined		bla_{VIM}
Yousefi et al (2010)[102]	Two hospitals in Orumieh and Tabriz	General inpatients	Different sites	*P. aeruginosa* (324)	✓	• DDST for detection of MBL • PCR	CIP 83% GEN 86% AMK 85%	• Resistance rate to MEM was 86% • Most of the isolates had an MIC ≥32 mcg/mL for IPM • Among nonsusceptible strains to IPM, 39 isolates were MBL positive • 18 out of resistant isolates carried bla_{VIM} and 6 isolates carried bla_{IMP}		• Large sample size	bla_{IMP} bla_{VIM}

(*Continued*)

Table 4 (*Continued*)

Author (year)	Setting	Population (no.)	Samples	Microorganism	MIC[a]	Method of carbapenemase enzyme detection	Resistance to FQ and AG	Outcome	Weak points	Strength points	*bla*-type genesc
Bahar et al (2010)[103]	One hospital in Tehran (Motahari)	Burn patients	Burn wounds	*P. aeruginosa* (186)	–	• Disk inhibitor synergy test • PCR	It was not reported for all isolates (only reported in MBL producers)	• EDTA disk showed that 23 strains produced MBL and all had bla_{VIM} gene • Mortality of MBL producer strains was 82% and non-MBL producer was 22%		• Large sample size • Mortality rates of MBL and non-MBL producers were reported	bla_{VIM}
Khosravi et al (2008)[104]	One hospital in Ahvaz	General inpatients	Different sites	*P. aeruginosa* (100)	–	• E-test for MBL detection • PCR	–	• Resistance rate to carbapenems was 41% • Of the resistant isolates, eight isolates were MBL producers and all contained bla_{VIM}	• FQ and AG were not tested		bla_{VIM}
Yazdi et al (2007)[105]	Two hospitals in Tehran	Not defined	Not defined	*P. aeruginosa* (126)	✓	• DD • E-test for MBL detection • PCR	–	According to MIC, resistance to IPM was 29% • 70 isolates were MBL producers • 8 isolates had bla_{VIM} • MIC of these eight strains was 32–64 mcg/mL for IPM	• Few antibiotics were tested	–	bla_{VIM}

Notes: [a]MIC values for carbapenems (but not other antimicrobial agents) were considered. [b]Resistance rates were according to the result of disk diffusion test. [c]Results about bla_{OXA-51} were not provided because this gene has no correlation with occurrence of resistance, unless it has *ISAba* genes in its upstream. [d]For complete information about the resistance to AG and FQ, please refer to the original reference.

Abbreviations: AG, aminoglycoside; AMK, amikacin; BA, boronic acid; CDT, combination disk test; CIM, carbapenem inactivation method; CIP, ciprofloxacin; DD, disk diffusion; DDST, double disk synergy test; DOR, doripenem; ERT, ertapenem; ESBL, extended spectrum β-lactamase; FQ, fluoroquinolones; GAT, gatifloxacin; GEN, gentamicin; ICU, intensive care unit; IPM, imipenem; KAN, kanamycin; KPC, *Klebsiella*-producing carbapenemase; LVX, levofloxacin; MBL, metallo-β-lactamase; MCNP, modified CarbaNP; MEM, meropenem; MHT, modified Hodge test; MIC, minimum inhibitory concentration; NAL, nalidixic acid; NOR, norfloxacin; OFX, ofloxacin; PCR, polymerase chain reaction; TOB, tobramycin; BAL, Broncho Alveolar Lavage.

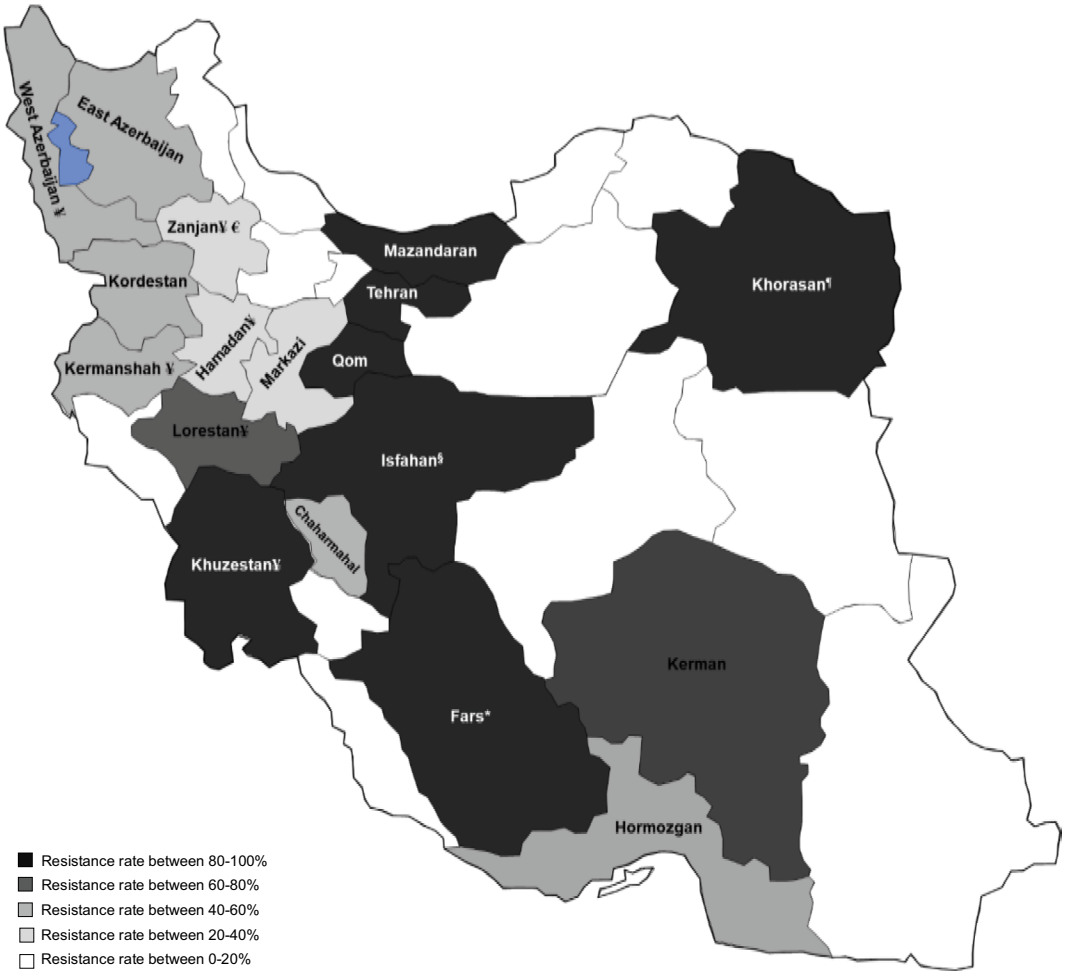

Figure 3 Carbapenem-resistance rates in different areas of Iran (according to the disk diffusion method).
Notes: The data were extracted from the latest available studies. Multicenter studies from different cities were not considered for mapping due to pooled data. The studies in the special populations (ie, pediatrics or cystic fibrosis) and outpatients were excluded. *Reported resistance rates from Fars province were conflicting (13.7% in 2017 and 96% in 2016). §Data for Isfahan province were obtained from both Kashan (a city around Isfahan) and Isfahan itself, although the sample size for Isfahan was very small. ¶Data for Khorasan area were extracted from a study in 2015 with 36 strains. €Intermediate and absolute resistance rates were 25% and 2%, respectively. ¥Data in these provinces were for 2010–2014 studies.

Most studies included biological samples from different sites like urine, trachea, and wounds. However, sites with highest or lowest resistant rates were not defined, except in a few studies. In study by Nobari et al, urine was the source with more resistant strains.[87] In the study by Khorvash et al, one *P. aeruginosa* strain that was isolated from urine samples harbored both bla_{IMP} and bla_{VIM} genes.[67]

Lack of clinical insight was the main limitation of almost all studies. Only in three studies, mortality rates in patients infected with resistant strains were addressed. Mortality rate of patients infected with carbapenem-resistant *K. pneumonia* isolates was 33% in 2013 (Rastegar Lari et al study).[25] In the same year, infections with carbapenem-resistant *A. baumannii* strains caused 20% mortality.[24] In the study by Bahar et al, the mortality rate of MBL-producer *P. aeruginosa* isolates vs non-MBL producers was 82% vs 22%.[103] In some studies, patients' conditions including requiring mechanical ventilation and days of hospital stay were considered, but no correlation between these data and acquiring carbapenem-resistant strains was found. Also in most studies, difference between infection and colonization was not clear.

Restrictions in technical facilities and standard laboratories in small cities are important issues. Access to a standard microbiological laboratory is not feasible in most regions. Disk diffusion was the main method for detecting carbapenem resistance until recently. Also, MIC breakpoints were adapted from CLSI and national data regarding these cutoffs are lacking. National antimicrobial resistance surveillance studies have not been well organized. Interpreting data from different regions of a country, considering the local standards, is essential.

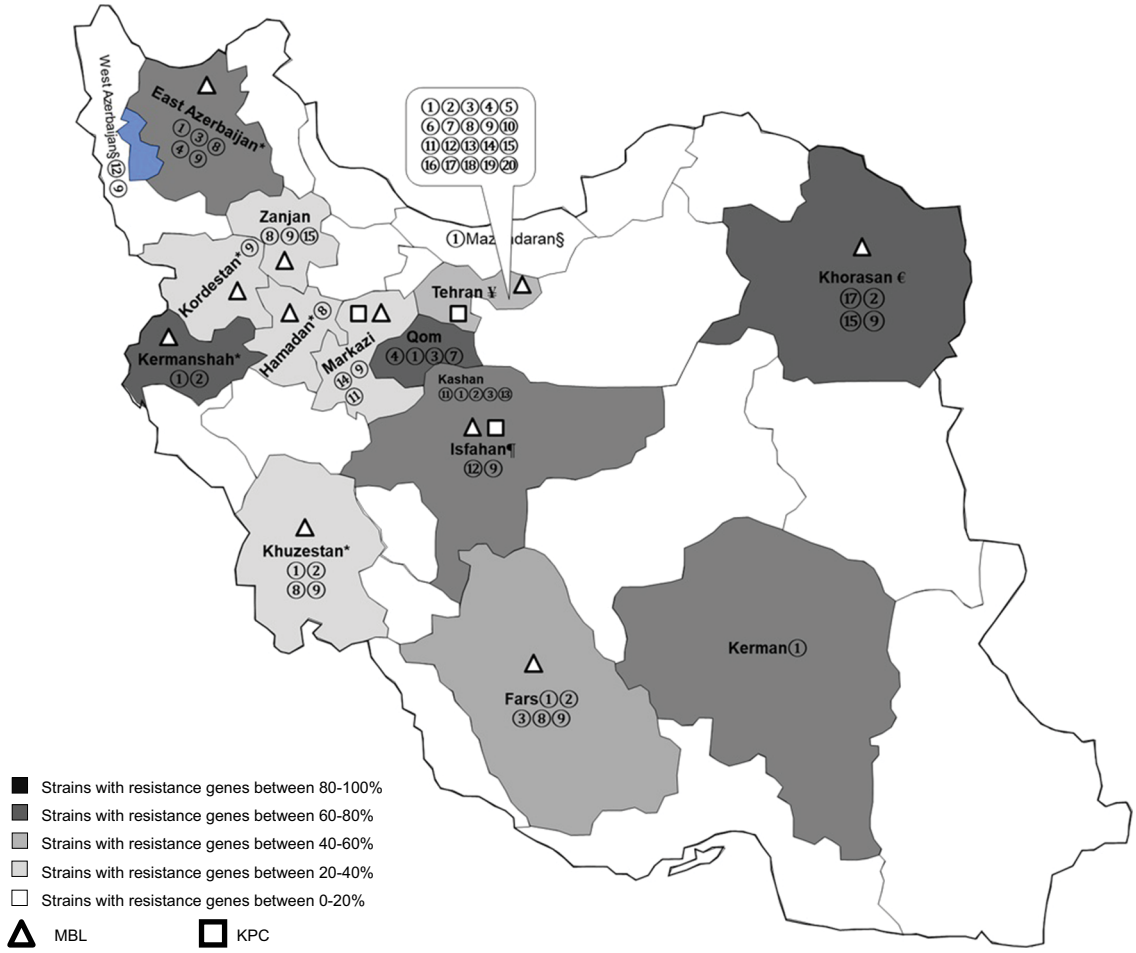

Figure 4 Carbapenem-resistant rates in different areas of Iran (according to the phenotypic and genotypic methods).

Notes: The data were extracted from the latest available studies. Multicenter studies from different cities were not considered for mapping due to pooled data. The studies in the special populations (ie, pediatrics or cystic fibrosis) and outpatients were excluded.

Some genes like blaSPM and blaSHV are mostly known as genes that encode ESBL enzymes, but they were included in this map, because these genes were assessed along with carbapenemase genes, and also overexpression of these genes concomitant with harboring efflux pump may be responsible for resistance to carbapenems. *Data for these five provinces were extracted from 2011 to 2014 articles, so new data are needed.

€Data were extracted from a 2015 study with 36 samples; another study in 2013 confirmed the presence of the genes of resistance in 4% of strains. ¥Data from Tehran province were conflicting; genes encoding resistance were detected in 15%–50% of isolates through 2015–2016. ¶Data from Kashan (a city in Isfahan province) showed presence of resistance genes in 80% of isolates in 2015.

§In Mazandaran and West Azerbaijan, these genes were reported, but rates of resistance were not included because the studies included different cities from different provinces. ①blaOXA-23 ②blaOXA-24 ③blaOXA-58 ④blaOXA-40 ⑤blaOXA-48 ⑥blaOXA-10 ⑦blaOXA-143 ⑧blaIMP ⑨blaVIM ⑩blaSPM ⑪blaGES ⑫blaPER ⑬blaNDM ⑭blaKPC ⑮blaTEM ⑯blaSHV ⑰blaADC ⑱blaSIM ⑲blaGIM.

Abbreviations: ESBL, extended spectrum β-lactamase; KPC, *Klebsiella*-producing carbapenemase; MBL, metallo-β-lactamase.

Pattern of antibiotic use can affect emergence of resistant microorganisms. Rational use of drugs, and specifically antibiotics, is a challenging issue in developing countries. Mean number of drugs per prescription in Iran was higher than the World Health Organization standards.[113,114] Approximately, 50% of inpatients, prescriptions contained at least one antibiotic, and this percentage was even higher in outpatient settings.[115] Overuse of antibiotics, especially injectable ones, and easy access to antibiotics without prescription is a warning alarm for future antibiotic resistance in developing countries. Establishing antimicrobial stewardship's programs is new in our hospitals. Unfortunately, governmental rules and supports to restrict antibiotic access in community pharmacies and prescription by general physicians are limited.

Following issues may be considered in future studies. Considering combination of antimicrobials for assessing the resistance is an important finding. Evaluating effects of combination disks (ie, a carbapenem with an aminoglycoside or a fluoroquinolone) on resistant gram-negative bacilli may be helpful.[116] Although combination therapy may increase the

Table 5 Types of bla genes, first location of isolation, and the relevant microorganisms in Iran

Ambler classes	Types of pf bla genes	First location of isolation	Year[a]	Total genes[b]	Microorganism[c]
A	GES	Tehran, by Shahcheraghi F.	2011	12	Mostly *P. aeruginosa*
	KPC	Tehran, by Nobari S.	2014	61	Mostly *K. pneumonia*
B	NDM	Tehran, by Shahcheraghi F.	2012	44	Mostly *K. pneumonia*
	VIM	Tehran, by Rezaei Yazdi H.	2007	437	Mostly *P. aeruginosa*
	IMP	Tabriz and Orumieh, by Yousefi S.	2010	271	Mostly *P. aeruginosa*
	SIM	Tehran, By Maspi H.	2016	2	Only in *A. baumannii*
	GIM	Tehran, By Maspi H.	2016	4	Only in *A. baumannii*
D	OXA-23	Tehran, by Taherikalani M.	2008	1,287	Only in *A. baumannii*
	OXA-24	Tehran, by Taherikalani M.	2008	213	Only in *A. baumannii*
	OXA-58	Tehran, by Taherikalani M.	2008	96	Only in *A. baumannii*
	OXA-40	Tabriz, by Sohrabi N.	2012	59	Only in *A. baumannii*
	OXA-48	Tehran, by Azimi L.	2014	52	In *K. pneumonia* and others
	OXA-10	Tehran, by Pakbaten S.	2015	12	Only in *P. aeruginosa*
	OXA-143	Qom, by Sharikhani Z.	2017	14	Only in *A. baumannii*

Notes: [a]Year that the genes were isolated for the first time. [b]Total number of genes that were reported in Iranian studies. [c]The microorganisms in which the genes were isolated.

risk of side effects, it remains an initial therapeutic option for MDR isolates. Another issue is studying of antibiotics' resistance pattern in health-care facilities. Remarkable increase in resistance rates among isolates from health-care facilities were reported. These strains may act as KPC-producing reservoirs.[117] A higher resistance rate in long-term care facilities in comparison with intensive care units has been identified.[118] Although some studies in Iran have included outpatients to evaluate carbapenem resistance, patients in long-term health-care facilities have not been included.

Authors' contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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