Antibiotic resistance of *Citrobacter freundii* in clinical isolates: A systematic review and meta-analysis

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Abstract

Introduction: *Citrobacter freundii* (*C. freundii*) is an opportunistic infection agent in hospitalized patients, especially in the intensive care units (ICUs). The prevalence of antibiotic resistance in *C. freundii* is increasing in the worldwide, and this may cause significant clinical problems. This paper aims at evaluating the rate of antibiotic resistance of *C. freundii* using meta-analysis.

Materials and methods: A total of eight qualified antibiotics in 21 published articles were chosen randomly to estimate the antibiotic resistance percentage of *C. freundii* in different countries. The data were analyzed by meta-analysis (random effect model) and the heterogeneity was determined using Cochran's Q and I^2 index. Also, Forest plot with confidence interval 95% were attained by R software.

Results: Our data showed that the antibiotic resistance pattern of *C. freundii* in different parts of the world was not absolutely same but the maximum range of resistance was related to gentamicin and the minimum range of resistance was related to imipenem. Q and I^2 were attained 45.25 ($P<0.05$) and 56%, respectively.

Conclusion: The findings of this study showed that the variability in antibiotic resistance pattern of *C. freundii* in different studies across the world was due to heterogeneity in hygiene level that resulted from various geographic regions.

Keywords: *Citrobacter freundii*, Antibiotic resistance, Nosocomial pathogen

Introduction

The genus *Citrobacter* is a gram negative, non-sporing rod which belongs to the family Enterobacteriaceae. It is emerging as important nosocomial pathogen. *Citrobacter freundii* (*C. freundii*) is an opportunistic pathogen that causes a range of nosocomial infections like respiratory tract, urinary tract, blood, septic arthritis, and many other normally sterile sites (1, 2). However, *C. freundii* has been isolated with resistance to multiple drugs, including extended spectrum beta- lactamases (ESBLs), new fluoroquinolones, and aminoglycosides in the worldwide (3, 4). The global emergence of multidrug-resistance (MDR) and extensively drug resistance (XDR) in the Entrobacteriaceae family like *C. freundii* are one of the key threats to human health (5).

Antimicrobial therapy for AmpC-producing Citrobacter may be hampered by the ability of bacteria to produce a wide variety of b-lactamases. The antibiotic agents that are available treatment for these bacteria are limited because these bacteria possess chromosomal AmpC beta-lactamases and produce ESBLs (6).

Materials and methods

The first step of data analysis was searching in notable databases such as Scopus, Pup Med, ISI Web of Science, Google Scholar, and CID using the keywords such: *C. freundii*, drug resistance, broad spectrum beta-lactamase (ESBL), AmpC, and Intensive Care Unit infection, the data were obtained. Also, there was no limitations time for collecting data. Therefore, among the collected articles the scales such as enough volume, considerable antibiotic, and access to the complete text of the related study were regulated. The extracted data assembled in table 1, which contains research location, antibiotics type surveyed, and percentage resistance of each one. Finally, appropriate studies were analyzed using statistical instruments. Many efforts were performed for collecting all of the noteworthy antibiotics, but all of these antibiotics were not investigated. Table1 contains the appropriate articles which selected for evaluated.

Table 1. The appropriate selected articles for estimate the percentage of antibiotic resistance (7-25).

Location	year	Volume	Ceftazidime	Ceftriaxone	Ciprofloxacin	Piperacillin	Piperacillin/tazobactam	Amikacin	imipenem	gentamicin	References
Taiwan	2000	61	57.4	57.4	32.8	70.5	-	26.2	0	55.8	7
America	2002	83	1.2	20.5	7.2	6.7	21	4.8	0	-	8
America	2004	75	6.7	14.7	1.3	4.7	14.7	4	0	-	8
America	2004	32	3.1	12.5	3.1	7.7	9.4	3.1	0	-	8
India	2010	40	70	57.5	67.5	80	30	-	7.5	70	9
Japan	2011	151	96.5	-	84.6	100	89.6	17.9	-	22.4	10
India	2012	49	67.35	48.98	71.43	30.61	6.12	30.61	4.08	67.35	11
India	2015	41	33	33	-	33	34	23	-	33	12
Iran	2015	60	20	16.7	16.7	-	-	-	0	6.7	13
India	2015	690	69	-	70	-	50	60	2	69	14
Iran	2016	400	70	57.5	67.5	80	30	0	7.5	70	15
India	2017	6	33.33	66.66	16.67	-	0	16.67	0	66.66	16
Greece	2017	173	23.7	23.1	8.1	-	22.5	2.3	1.2	3.5	17
Bangalore	2016	5	69	-	70	-	50	60	2	69	18
China	2017	1	29	6	13	-	-	0	0	2	19
Iran	2013	13	28.3	39.5	27.5	51.7	-	1.25	0	18.3	20
Iran	2018	21	66.6	47.7	76.2	-	-	19	4.8	28.5	21
Iran	2013	31	15	15	25	-	-	7	2	65	22
Italy	2013	17	100	-	0	-	100	12.5	25	100	23
Japan	2011	66	95.5	-	83	-	-	17	1	33	24
India	2012	6	69.39	48.96	71	-	-	30.61	4.8	67	25

Statistical analysis

The results were reported as percentage ± standard deviation (SD) for resistance antibiotics. To evaluate the heterogeneity, Cochran's test and I^2 index were applied to quantify the depression in the meta-analysis. All the statistical methods were done by R software, version 3.4.1 with Meta Package in the level significance less than 0.05.

Results

Totally, 21 mentioned articles were chosen to estimate the antibiotic resistance percentage (see Table 1). Because the antibiotics were not present for evaluations in some studies, the resistance percentage

for total studies were calculated based on the weighted mean of resistance percentages. Table 2 contains the information about antibiotics resistance percentage and its standard deviation. Based on this table, the maximum resistance percentage was related to gentamicin with 0.49 ± 0.11 and the minimum range of resistance was belonged to imipenem with 0.023 ± 0.04.

Table 2. The data are related to antibiotics resistance percentage along with percentage ± standard deviation.

Antibiotic	Percentage ± Standard deviation
Ceftazidime	0.42 ± 0.11
Ceftriaxone	0.35 ± 0.12
Ciprofloxacin	0.41 ± 0.11
Piperacillin	0.36 ± 0.16
Piperacillin/Tazobactam	0.35 ± 0.13
Amikacin	0.18 ± 0.09
Imipenem	0.023 ± 0.04
Gentamicin	0.49 ± 0.11

Figure 1 depicts the forest plot along with Cochran's Q and I^2 index were used to analysis of the data. Middle resistance points in each segment were estimated by percentage with confidence interval 95%. It can be seen that Q and I^2 are attained 45.25 (P = 0.000) and 56%, respectively. These results showed that most of the variability across studies is due to heterogeneity. Moreover, the weighted average of each article was achieved by a random effects meta-analysis.

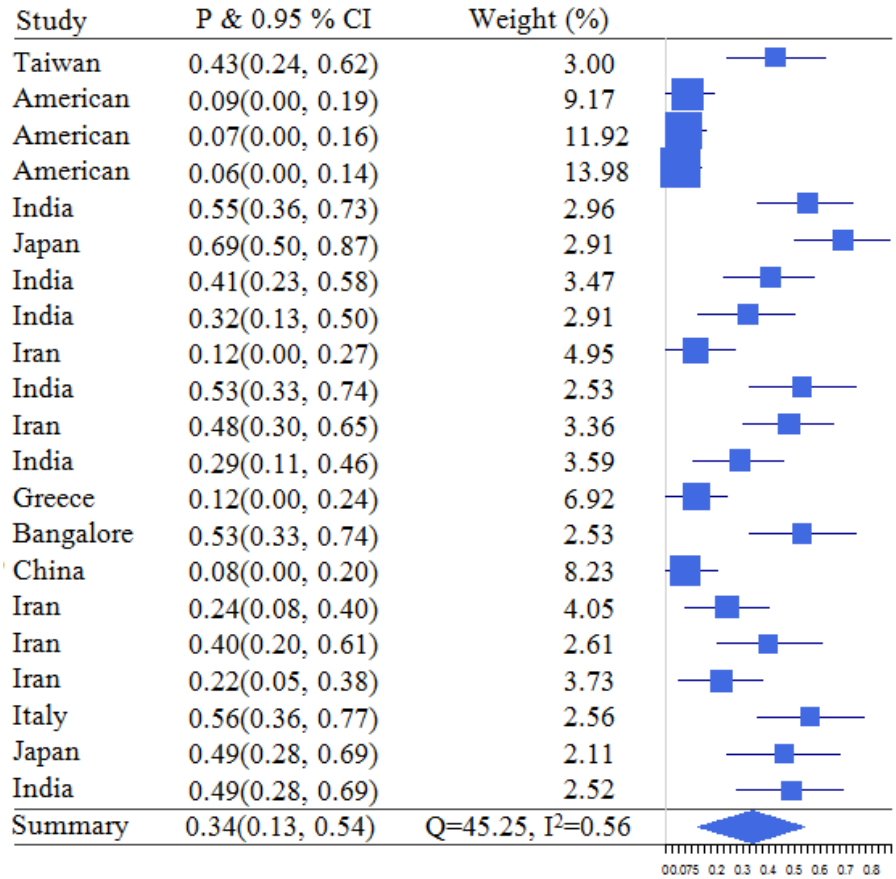

Figure 1. Forest Plot for the data in terms of percentage along with heterogeneity statistics.

Discussion

Since the emergence of beta-lactamases traced back nearly 60 years ago, they have consistently been the most widely used antibiotics, currently accounting for 50% of the antibiotics consumed globally (26). The most common beta-lactamases among the *Entrobacteriaceae* are the plasmid borne class TEM and SHV beta-lactamases. TEM-1, first reported in 1965, confers a high level of resistance to penicillins and early cephalosporins but little resistance to oxyiminocephalosporins and aztreonam (27). Widespread resistance to penicillin stimulated the development of chemically modified beta-lactams, including cephalosporins and monobactams, which were less sensitive to hydrolysis by the class beta-lactamases. Beginning in the early 1980s, extended-spectrum beta-lactamases (ESBL) derived from TEM-1 and SHV-1 began to appear in response to the widespread use of cephalosporins that had occurred in the previous decade. Indeed, with the exception of the Carbapenem, as newer variants of beta–lactam antibiotics have been introduced; TEM variants active against those beta-lactams have appeared within 2 to 3 years (27). Based on our calculations, the maximum and minimum ranges of antibiotic resistance were related to gentamicin and imipenem, respectively. In the present study, by reviewing the related reports among the data, the authors observed heterogeneity among the antibiotic resistance percentages across different countries. Multidrug-resistant *C.freundii* has been associated with a higher rate of nosocomial infectious mortality compared to susceptible strains (28, 29). Also, it was justified that the antibiotic resistance is depended on various geographic regions. Wickwire et al. (2003) suggested treatment for *Citrobacter* infections including Carbapenem (Imipenem + meropenem, cilastatin) as the first choice and fluoroquinolones (moxifloxacin, ciprofloxacin, gatifloxacin, levofloxacin, lomefloxacin) with anti-pseudomonas aminoglycosides (Gentamicin and Tobramycin) are alternation for an adult patient (29).Therefore, rapid detection of existence resistance in *C. freundii* is demonstrated that which kind of timely appropriate therapy is necessary to control the initiate effective infection (30). Finally, we suggest that the more important antibiotic resistance contains AmpC, ESBL, and carbapenemase phenotypes which have the most common resistance mechanism need for more studies.

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