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

Arman Rostamzad¹, Abozar Karami Cherag Abad¹, Mehdi Omidi^{2*}, Ali Asghar Hatamnia¹

- 1. Department of Biology, Faculty of Basic Science, Ilam University, Ilam, Iran
- 2. Department of Mathematics, Faculty of Basic Science, Ilam University, Ilam, Iran

*Corresponding author:Tel: +98 9388986741 Fax: +98 -

Address: Department of Mathematics, Faculty of Basic Science, Ilam University, Ilam, Iran

E-mail: m.omidi@ilam.ac.ir

Received; 16/01/2019 revised; 27/02/2019 accepted; 8/05/2019

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 I² 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 I² 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 multidrugresistance (MDR) and extensively drug resistance (XDR) in the Entrobacteriaceae family like *C. freundii* are one of the key threats to human health (5).

 $\begin{tabular}{ll} \textbf{Copyright} @ \textbf{2019 Journal of Basic Research in Medical Science.} & This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (\begin{tabular}{ll} \https://creativecommons.org/licenses/by-nc/4.0/ \end{tabular}) which permits copy and redistribute the material, in any medium or format, provided the original work is properly cited. \\ \end{tabular}$

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	Volu me	Ceftazi dime	Ceftria xone	Ciprof loxacin	Piperacil lin	Pipera cillin/t azobac tam	Amika cin	imipe nem	gentamici n	Referenc es
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 \pm standard deviation (SD) for resistance antibiotics. To evaluate the heterogeneity, Cochran's test and I² 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 \pm 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 I² 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 I² 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.

Study	P & 0.95 % CI	Weight (%)	
Taiwan	0.43(0.24, 0.62)	3.00	-
American	0.09(0.00, 0.19)	9.17	-
American	0.07(0.00, 0.16)	11.92	
American	0.06(0.00, 0.14)	13.98	
India	0.55(0.36, 0.73)	2.96	
Japan	0.69(0.50, 0.87)	2.91	
India	0.41(0.23, 0.58)	3.47	-
India	0.32(0.13, 0.50)	2.91	
Iran	0.12(0.00, 0.27)	4.95	-
India	0.53(0.33, 0.74)	2.53	
Iran	0.48(0.30, 0.65)	3.36	
India	0.29(0.11, 0.46)	3.59	_
Greece	0.12(0.00, 0.24)	6.92	-
Bangalore	0.53(0.33, 0.74)	2.53	
China	0.08(0.00, 0.20)	8.23	-
Iran	0.24(0.08, 0.40)	4.05	_
Iran	0.40(0.20, 0.61)	2.61	_
Iran	0.22(0.05, 0.38)	3.73	-
Italy	0.56(0.36, 0.77)	2.56	_
Japan	0.49(0.28, 0.69)	2.11	
India	0.49(0.28, 0.69)	2.52	_
Summary	0.34(0.13, 0.54)	Q=45.25, I ² =0.56	

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 1980s, extended-spectrum betalactamases (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 Carbapenem, as newer variants of betalactam antibiotics have been introduced; TEM variants active against those betalactams have appeared within 2 to 3 years (27). Based on our calculations, maximum and minimum ranges of

References

- 1. Whalen JG, Mully TW, English J C. Spontaneous Citrobacter freundii infection in an immunecompetent patient. J Arch Dermatol. 2007; 143(1):124-5. doi: 10.1001/archderm.143.1.124.
- 2. Bruehl CL, Listernick R. Citrobacter freundii septic arthritis. J Paediatr Child Health.1992; 28(5):402-3. doi:10.1111/j.1440-1754.1992.tb02701.x.

antibiotic resistance were related gentamicin and imipenem, respectively. In the present study, by reviewing the related reports among the data, the authors heterogeneity observed 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 depended various is on 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 antipseudomonas aminoglycosides (Gentamicin Tobramycin) and 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.

- 3. Gootz TD, Jackson DB, Sherris JC. Development of resistance to cephalosporins in clinical strains of Citrobacter sp. J Antimicrob Agents Chemother.1984; 25:591-5.
- 4. Aoyama H, Fujimaki K, Sato K, Fujii T, Inoue M, Hirai K, et al. Clinical isolate of Citrobacter freundii highly resistant to new quinolones. Antimicrob Agents Chemother. 1988; 38:922-4.

- 5. Magiorakosa P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant international bacteria: an expert for interim standard proposal definitions for acquired resistance. J Clin Microbiol Infect. 2012; 18(3): 268-10.1111/j.1469-81. doi: 0691.2011.03570.x.
- Choi SH, Lee JE, Park SJ, Kim MN, Choo EJ, Kwak YG, et al. Prevalence, microbiology, and clinical characteristics of extended-spectrum blactamase-producing Enterobacterspp., Serratia marcescens, Citrobacter freundii, and Morganella morganii in Korea. Eur J Clin Microbiol Infect Dis. 2007; 26: 557-61. doi: 10.1007/s10096-007-0308-2.
- 7. Wang JT, Chang SC, Chen YC, Luh KT. Comparison of antimicrobial susceptibility of Citrobacter freundii isolates in two different time periods. J Microbiol Immunol Infect. 2000; 33(4):258-62.
- 8. Shawn R, Lockhart, Murray A, Abramson SE, Beekmann GG, Riede S, Diekema DJ, Quinn J P, Doern G V. Antimicrobial resistance among gramnegative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. J Clin Microbiol. 2007; 45(10):3352-9. doi: 10.1128/JCM.01284-07.
- 9. Shahid M, Citrobacter spp. Simultaneously Harboring blaCTX-M, blaTEM, blaSHV, blaampC, and Insertion Sequences IS26 and orf513: An evolutionary phenomenon of recent concern for antibiotic resistance. J Clin Microbiol. 2010; 48(5):1833-9. doi: 10.1128/JCM.01467-09.
- 10. Kanamori H, Yano H, HirakataY, Endo SH, Arai K, Ogawa M, et al. prevalence of extended-spectrum b-lactamases and qnr determinants in Citrobacter species from Japan: dissemination of CTX-M-2. J Antimicrob Agents Chemother.

- 2011; 66:2255-62. doi: 10.1093/jac/dkr283.
- 11. Khanna A, Singh N, Aggarwal A, Khanna M. The antibiotic resistance pattern in Citrobacter species: An emerging nosocomial pathogen in a tertiary care hospital. J CDR. 2012; 6(4):642-4.
- 12. Mishra MP, Sarangi R, Padhy RN. Prevalence of multidrug resistant uropathogenic bacteria in pediatric patients of a tertiary care hospital in eastern India. J Infect Public Health. 2016; 9(3):308-14.doi: 10.1016/j.jiph.2015.10.002.
- 13. Akya A, Jafari S, Ahmadi K, Azam Elahi A. [Frequency Of blaCTX-M, blaTEM and blaSHV genes in Citrobacters isolated from Imam Reza hospital in Kermanshah]. J Mazandaran Univ Med Sci. 2015; 25 (127):65-73 (Article in Persian)
- 14. Kasukurthy P, Rani L, Ramaswam R. Isolation and antibiotic sencitivity pattern of Citrobacter species with ESBL and AMPC detection at tertiary care hospital, Bangalor. J Evolution Med. Dent. Sci. 2016; 5(30):1553-6. doi:10.14260/jemds/2016/365.
- 15. Taghi M, Najafi Nasab E, Ghotaslou R, Pirzadeh T, Asgharzadeh M, Gholizadeh P, et al. Prevalence of ESβL types TEM, SHV,CTX in isolates of Salmonella, Citrobacter and Enterobacter spp. from patients feaces in Tabriz. IJMRHS. 2016; 5(9):197-204.
- 16. Bajaj A, Kukanur S. Multidrug resistant gram negative bacilli causing surgical site infections: isolation and antimicrobial susceptibility. Int J Curr Microbiol App Sci. 2017; 6(4):2244-55. doi:10.20546/ijcmas.2017.604.261.
- 17. Maraki S, Vardakas KZ, Mavromanolaki VE, Margarita Kyriakidou, George Spais, Diamantis P, et al. In vitro susceptibility and resistance phenotypes in contemporary Citrobacter isolates in a university

- hospital in Crete, Greece. Infect Dis. 2017; 1(1):1-8.
- 18. Priyadarshini P, Leela Rani K, Ramaswamy R. Isolation and antibiotic sensitivity pattern of Citrobacter species with ESBL and AMPC detection at tertiary care hospital, Bangalor. J Evolution Med. Dent. Sci.2016; 5(30):1553-7. doi:10.14260/jemds/2016/365.
- 19. Liu L, Lan R, Liu L, Wang Y, Zhang Y, Wang Y, et al. Antimicrobial resistance and cytotoxicity of Citrobacter Spp. in Maanshan Anhui province, China. Frontiers in Microbiology. 2017; 8(1357):1-12. doi:10.3389/fmicb.2017.01357.
- 20. Mousavian SM, AhmadKhosravi Shoja S. Survey of frequency in extended-spectrum beta-lactamase producing entrobacteriaceae determination of the antibiotic resistant pattern in clinical specimens in teaching hospitals of Ahvaz Jundishapur university of medical sciences. Jundishapur Sci Med J. 2014; 13(2):1-9.
- 21. Jasemi SS, Alipoor F, Dehbashi S, Mardaneh J. [Isolation of Citrobacter spp. from blood specimens in patients hospitalized in Kermanshah Imam Khomeini hospital and determination of the of isolates sensitivity to antibiotics]. J Birjand Univ Med Sci. 2014; 21(3):394-400. (Article in Persian)
- 22. Shokri D, Mobasherizadeh S, Norouzi Baruq M, Yaran M. Isolation and identification of carbapenemase KPC producing strains of entrobacteriaceae and determination of their antibiotic susceptibility patterns. JIUMS. 2013; 31(248):1247-56.
- 23. Gaibani P, Ambretti S, Farruggia P, Bua G, Berlingeri A, Vittoria M, et al. Outbreak of Citrobacter freundii carrying VIM-1 in an Italian hospital, identified during the carbapenemases

- screening actions, Int J Infect Dis. 2012; 17 (2013):e714–e7. doi:10.1016/j.ijid.2013.02.007.
- 24. Kanamori H, Yano H, Hirakata Y, Endo Sh, Arai K, Ogawa M, et al. High prevalence of extended-spectrum blactamases and qnr determinants in citrobacter species from Japan: dissemination of CTX-M-2. J Antimicrob Chemother. 2011; 66(1): 2255-62. doi: 10.1093/jac/dkr283.
- 25. Khanna A, Singh N, Aggarwal A, Khanna M. The antibiotic resistance pattern in Citrobacter species: An emerging nosocomial pathogen in a tertiary care hospital. JCDR. 2012; 6(4):642-4.
- 26. Ades AE, Lu G, Higgins JP.The interpretation of random-effects meta-analysis in decision models. Med Decis Making. 2005; 25(6):646-54. doi:10.1177/0272989X05282643.
- 27. Livermore, D M. Are all beta-lactams created equal? J Scand J Infect Dis Suppl.1996; 101(1):33–43.
- 28. Medeiros AA. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. Clin. Infect. Dis.1997; 24(1):19-45. doi:10.1093/clinids/24.supplement_1.s
- 29. Deal EN, Micek ST, Ritchie DJ, Reichley RM, Dunne M, Kollef MH. Predictors of in-hospital mortality for bloodstream infections caused by Enterobacter species or Citrobacter freundii. Pharmacotherapy. 2007; 27(2):191-9. doi:10.1592/phco.27.2.191.
- 30. Wickwire C, Gilbert D, Moellering, RC, Sande MA. The sanford guide to antimicrobial therapy, Edition 33. Jeb C. Sanford. Antimicrobial Therapy Inc., Hyde Park, VT, 2003.