He et al. Ann Clin Microbiol Antimicrob (2017) 16:32
DOI 10.1186/s12941-017-0207-z

Annals of Clinical Microbiology and Antimicrobials

RESEARCH

Open Access

Molecular characteristic of *mcr*-1 producing *Escherichia coli* in a Chinese university hospital

Qing-wen He[1,2†], Xiao-hong Xu[1†], Fang-jun Lan[1,2], Zhi-chang Zhao[3], Zhi-yun Wu[1,2], Ying-ping Cao[1] and Bin Li[1*]

Abstract

Background: Colistin has been considered as a last-line treatment option in severe infections caused by multidrug-resistant (MDR) gram-negative pathogens. However, the emergence of the mobile colistin resistance gene (*mcr*-1) has challenged this viewpoint. The aim of this study is to explore the prevalence of *mcr*-1 in *Escherichia coli* (*E. coli*) in a Chinese teaching hospital, and investigate their molecular characteristics.

Methods: A total of 700 *E. coli* isolates were used to screen *mcr-1* by PCR and sequencing in a Chinese university hospital from August 2014 to August 2015. Susceptibility test of *mcr*-1-producing isolates was determined by Vitek -2 Compact system. 26 virulence factors (VFs), phylogenetic groups, Multi-locus sequence typing (MLST), and DNA Fingerprinting (ERIC-PCR) of strains were investigated by PCR.

Results: Four (0.6%) *mcr*-1 producing *E. coli* isolates were found in this study. The results of antibiotic susceptibility test showed that all four isolates were resistant to colistin, ciprofloxacin, levofloxacin, cefazolin, and trimethoprim/sulfamethoxazole, and were susceptible to amikacin, ertapenem and imipenem. In addition, all 4 isolates exhibited high-level resistance to aztreonam, cefotaxime and gentamicin. The numbers of VFs contained in *mcr*-1 positive isolates were no more than 4 in our study. MLST result demonstrated that these isolates were assigned to two sequence types: ST156 and ST167. The result of phylogenetic analysis showed that four *mcr*-1-positive isolates belong to two phylogenetic groups: A and B1 group. ERIC-PCR showed that four *mcr*-1 positive strains were categorized into three different genotypes.

Conclusions: Our study demonstrated a low prevalence of *mcr*-1 in *E. coli* clinical isolates in a Chinese teaching hospital, and we have gained insights into the molecular characteristics of these *mcr*-1-positive strains. Increasing the surveillance of these infections, as well as taking effective infection control measures are urgently needed to take to control the transmission of *mcr*-1 gene.

Keywords: *E. coli*, *mcr*-1, Colistin, Multidrug-resistant

Background

In recent years, colistin has been considered as an effective therapeutic option for the rapid increasing of multidrug-resistant (MDR) gram-negative pathogens [1, 2]. However, the prevalence of the mobile colistin resistance gene (*mcr*-1) in animals and human beings worldwide has challenged this viewpoint [3, 4]. Resistance to polymyxins is mainly caused by the modification to bacterial outer membrane, which was usually considered as chromosomally mediated resistance [5, 6].

Since it was initially found, plasmid-mediated *mcr*-1 has been detected widely [3, 7]. Nowadays, *mcr*-1-producing bacteria have been reported in many regions in China [4, 8]. *Mcr*-1 was firstly found in *Escherichia coli* (*E. coli*), and now it has been spreading to other *Enterobacteriaceae* [9]. Several reports showed that the *mcr*-1 gene could coexist with other resistance genes (such as

*Correspondence: leonlee307@hotmail.com
†Qing-wen He and Xiao-hong Xu contributed equally to this work
[1] Department of Clinical Laboratory, Fujian Medical University Union Hospital, 29# Xinquan Road, Fuzhou 350001, Fujian, China
Full list of author information is available at the end of the article

CRE/ESBL) in *E. coli* and *Klebsiella pneumoniae*, which probably lead to the emergence pan-drug resistant and increase the difficulty of treatment [8, 10]. Therefore, the emergence and spread of *mcr*-1 gene among human beings should be given close attention. The aim of this study was to evaluate the prevalence of *mcr*-1 in *E. coli* clinical isolates in a Chinese teaching hospital, and to investigate the molecular characteristics of these strains.

Methods

Bacterial strains

A total of 700 *E. coli* clinical isolates were collected from the clinical laboratory of Fujian Medical University Union Hospital (Fuzhou, Fujian province, China) from August 2014 to August 2015. It is a 2200-bed tertiary care teaching hospital with approximately 95,000 hospital admissions per year, located in southeastern China. All isolates were identified by GNI card of the Vitek system (BioMèrieux, Missouri, France).

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using the AST-GN16 of Vitek-2 Compact system (Bio Mérieux, France). The antimicrobial agents tested included: tigecycline (glycycline); ertapenem and imipenem (carbapenems); cefazolin; cefoxitin; cefepime, and cefotaxime (cephalosporins); aztreonam (monobactam); amikacin and gentamicin (aminoglycosides); ciprofloxacin and levofloxacin (quinolone); piperacillin/tazobactam; trimethoprim/sulfamethoxazole. The results were interpreted by the Clinical and Laboratory Standards Institute (CLSI) [11]. The MIC of colistin was determined using agar dilution method, and the result was interpreted according to European Committee On Antimicrobial Susceptibility Testing (EUCAST) guidelines [12]. *E. coli* ATCC 25922 was used as a quality control.

DNA extraction

Several colonies were suspended in 50 µl of sterile distilled water for preparing genomic DNA of the isolates, and then the bacterial suspension was heated at 100 °C for 10 min as described previously [13].

MCR-1 detection

mcr-1 gene was screened in *E. coli* clinical isolates by PCR using primers as previously described [4]. All of the PCR products were sequenced and then compared with known sequences listed in the GenBank (http://www.ncbi.nlm.nih.gov/blast/).

Detection of virulence factor genes

Twenty six virulence factors (VFs) genes associated with extraintestinal virulence [14, 15] were detected using a multiplex PCR method as previously described [15]. These genes were as follows: adhesions (*pap*AH, *pap*EF, *pap*C, *pap*G allele I, *pap*G II/III, *pap*G allele II, *sfa/foc*DE, *afa/dra*BC, *fim*H, *gaf*D, *sfa*S, *foc*G and *nfa*E), toxins (*hly*A, *cnf*1 and *cdt*B), siderophores (*fyu*A and *iut*A), protections and invasions (*kps*MTII, *kps*MTIII, *tra*T, *cva*C, kpsMT and K1/K5), miscellaneous (*rfc* and PAI). The PCR products were sequenced and then compared with known sequences listed in the GenBank (http://www.ncbi.nlm.nih.gov/blast/).

Phylogenetic analysis

The phylogenetic groups (A, B1, B2, and D) of *mcr*-1 producing *E. coli* isolates were identified by a triplex PCR as previously described [16].

Multi-locus sequence typing (MLST)

Mcr-1 positive strains were analyzed by multilocus sequence typing (MLST), which was based on 7 standard housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA, recA*) (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli) [17].

DNA fingerprinting

Enterobacterial Repetitive Intergenic Consensus Sequences PCR (ERIC-PCR) was applied to typing *mcr*-1 producing *E. coli* isolates with the primers ERIC-1 and ERIC-2 [18]. DNA fingerprints were compared by visual inspection, ERIC profiles were regarded as different if there were different bands on visual inspection [19].

Results and discussion

In this study, four isolates (0.6%) were confirmed to carry *mcr*-1 gene, which is lower than previous study [4]. The age of the patients ranged between 38 and 80 years. These *mcr*-1 producing strains were isolated from two different wards (Table 1). Two strains were isolated from the same patient. The clinical data of patients with *mcr*-1 positive *E. coli* infection were shown in Table 2.

Mcr-1 was usually found to be co-localized with other resistance genes on plasmids, such as ESBL genes and carbapenemase genes [20], which might increase the emergence of pan-drug resistance. In our study, the results of antimicrobial susceptibility test showed a high drug resistance in the *mcr*-1-producing isolates. All of the *mcr*-1 positive isolates were resistance to at least 3 different kinds of antibiotics (Table 1).

All four *mcr*-1 positive strains detected in our study were resistant to colistin and the MICs ranged from 4 to 16 µg/ml. It will be worrisome once *mcr*-1 coexists with other resistant genes, especially carbapenemase genes because of limited therapeutic options [20]. Previous studies revealed that *mcr-1* co-produced with carbapenem-resistant genes in *E. coli* [8, 21]. Fortunately, all of

Table 1 Main characteristics of the *mcr*-1 *E. coli*

Isolates	Data	Ward	Specimen	Phylogenetic groups	MLST	ERIC pattern	VFs	Antibiotic resistance
E321	2014.8	Colorectal surgery	Drainage-fluid	B1	ST156	1	*traT*, *iutA*	COL, CFZ, FOX, CIP, LVX, SXT, TGC
E684	2015.1	Colorectal surgery	Secretion	B1	ST156	2	*fimH*, *traT*, *iutA*	COL, CFZ, CTX, FEP, ATM, GEN, CIP, LVX, SXT
E921	2015.4	Hepatobiliary surgery	Secretion	A	ST167	3	*fyuA*, *traT*, *iutA*	COL, CFZ, CTX, ATM, GEN, CIP, LVX, SXT
E1005	2015.5	Hepatobiliary surgery	Drainage-fluid	A	ST167	3	*fyuA*, *cvaC*, *traT*, *iutA*	COL, CFZ, CTX, ATM, GEN, CIP, LVX, SXT

CFZ cefazolin, *FOX* cefoxitin, *CTX* cefotaxime, *FEP* cefepime, *TZP* piperacillin/tazobactam, *ATM* aztreonam, *IPM* imipenem, *ETP* ertapenem, *AMK* amikacin, *GEN* gentamicin, *CIP* ciprofloxacin, *LVX* levofloxacin, *SXT* trimethoprim/sulfamethoxazole, *TIG* tigecycline, *COL* colistin, *MLST* multi-locus sequence typing, *ERIC* enterobacterial repetitive intergenic consensus, *VFs* virulence factor genes

Table 2 Clinical data of patients with mcr-1 positive *E. coli* infection

Isolates	Patients	Gender	Age (years)	Underlying diseases	Length of hospital stay (days)	Treatments used	Outcomes
E321	Patient 1	Female	80	Malignancy, hypertension, pulmonary tuberculosis	51	TZP	Survived
E684	Patient 2	Female	57	Perineal infection, hypertension	37	TZP	Survived
E921	Patient 3	Male	38	Hypertension, pancreatitis, diabetes	26	MEM	Survived
E1005	Patient 3	Male	38	Hypertension, pancreatitis, diabetes	26	MEM	Survived

TZP piperacillin/tazobactam, *MEM* meropenem

them were susceptible to carbapenems (IPM and ETP), which probably indicated that no carbapenem-resistant genes coexisted with *mcr*-1. Result of ERIC-PCR (Fig. 1) showed that four *mcr*-1 positive strains were categorized into three different genotypes, one of which contained 2 strains (from the same patient). These isolates which have different patterns suggest that they were non-clonal transmission. In a previous study, two *mcr*-1 positive *E. coli* isolates from a single fowl were belonging to phylogenetic B1 and D group [22]. The *mcr*-1-producing isolates in this study were belonged to phylogenetic groups A and B1, which were mainly distributed among human commensal *E. coli* isolates [23]. The *mcr*-1 producing isolates were assigned by MLST to two different sequence types: ST156 and ST167 (Table 1), which was similar to previous reports in other studies from China [8, 22]. *E. coli* ST156 has been found that it has connection with different ESBL genes [24, 25]. ST167 was belonged to ST10 complex and regarded as prevalent ST among ESBL-producing *E. coli* from human and animal sources [26]. In addition, *E. coli* ST167 was reported to be closely related to bla_{NDM}, which needed closely concern of spreading [27]. The similar molecular characterizations illustrated that *mcr*-1 positive isolates detected from the same department in our study were clonally related.

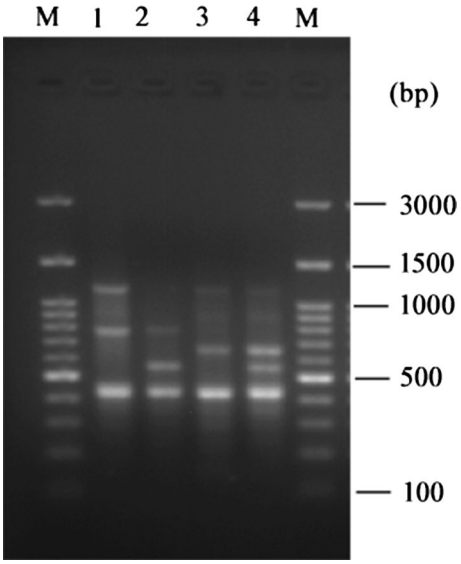

Fig. 1 ERIC-PCR products from four mcr-1 positive isolates. *M* mark, *lane 1* E321, *lane 2* E684, *lane 3* E921, *lane 4* E1005

VFs in *E. coli* were associated with colonization, bacterial fitness and virulence [28]. VFs include five main groups: (1) adhesions; (2) toxins; (3) siderophores; (4) capsule production and (5) protections and invasions.

Clinical *E. coli* strains often carry multiple VFs, and isolates belonging to groups A and B1 often have less VFs than those belonging to phylogroups B2 and D [28]. To the best of our knowledge, there is no study concerning about VFs in *mcr-1* producing *E. coli*. In our study, *mcr-1* producing isolates contained less than 4 different VFs (Table 1). Only five different kinds of VFs had been detected in our *mcr-1* positive isolates, which included *fim*H, *fyu*A, *tra*T, *iut*A and *cva*C. *fim*H is one of the most commonly VFs present in *E. coli*, which encodes the adhesion subunit of type 1 fimbriae and related to colonization [15]. Lee et al. reported that *fyu*A, *tra*T, and *iut*A were found to be independent predictors for pathogenicity. Meanwhile, *tra*T and *iut*A were thought to be closely related to ESBL genes [29]. Pitout et al. found that *cva*C was only present in non-CTX-M-producing isolates [30]. Previous reports suggested that antibiotic resistance has negative association with virulence factors [31], which could be interpreted by the loss of VFs associated with mutation to resistance [32].

It is noteworthy that two *mcr*-1 positive *E. coli* strains were isolated from the same patient but at different time (Table 1). Results of MLST and ERIC-PCR revealed that these isolates had identical genetic background. Result of antimicrobial susceptibility test showed that they had similar antibiograms. We speculate that the two isolates probably originated from a same source.

In conclusion, we have revealed a low prevalence of *mcr-1* in *E. coli* clinical isolates in a Chinese teaching hospital, and presented detailed molecular characteristics of these isolates. The presence of *mcr*-1 in *E. coli* clinical isolates suggests that it will pose a threat to public healthcare. Effective infection control measures are urgently needed to take to control the transmission of *mcr*-1 gene.

Abbreviations

MCR-1: mobile colistin resistance gene; MDR: multi-drug resistant; PCR: polymerase chain reaction; CLSI: Clinical and Laboratory Standards Institute; MLST: multi-locus sequence typing; CRE: carbapenem-resistant *Enterobacteriaceae*; ESBL: extended-spectrum β-lactamase; ERIC-PCR: Enterobacterial Repetitive Intergenic Consensus Sequences PCR; VFs: virulence factors.

Authors' contributions

QH, XX, FL, ZZ and YC conducted laboratory assays. ZW collected clinical data. QH wrote the paper. BL designed the study and reviewed the manuscript. All authors read and approved the final manuscript.

Author details

[1] Department of Clinical Laboratory, Fujian Medical University Union Hospital, 29# Xinquan Road, Fuzhou 350001, Fujian, China. [2] The Union Clinical Medical College of Fujian Medical University, Fuzhou 350004, Fujian, China. [3] Department of Pharmacy, Fujian Medical University, Union Hospital, Fuzhou 350001, Fujian, China.

Acknowledgements

We thank Prof. Jianhua Liu and Yiyun Liu of South China Agricultural University, Guangzhou, China for kindly providing us the mcr-1 positive control. We also thank Prof. Min Chen and Department of Laboratory Medicine, Medical Technology and Engineering College, Fujian Medical University for their kindly help.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

There is no additional data and materials, except those in the sections of "Methods" and "Results and discussion".

Funding

This study was supported by the National Natural Science Foundation of China (Grant No. 81201328), the Fujian Provincial Funds for Distinguished Young Scientists in Colleges and Universities, China (Grant No. JA13134), and the Medical Elite Cultivation Program of Fujian, China (Grant No. 2015-ZQN-ZD-15).

Publisher's Note

Received: 16 October 2016 Accepted: 8 April 2017
Published online: 19 April 2017

References

1. Nation RL, Li J, Cars O, Couet W, Dudley MN, Kaye KS, Mouton JW, Paterson DL, Tam VH, Theuretzbacher U, et al. Framework for optimisation of the clinical use of colistin and polymyxin B: the Prato polymyxin consensus. Lancet Infect Dis. 2015;15(2):225–34.
2. Doi Y, Paterson DL. Carbapenemase-producing Enterobacteriaceae. Semin Respir Crit Care Med. 2015;36(1):74–84.
3. Skov RL, Monnet DL. Plasmid-mediated colistin resistance (mcr-1 gene): 3 months later, the story unfolds. Euro Surveill. 2016;21:9.
4. Liu YY, Wang Y, Walsh TR, Yi L, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16(2):161–8.
5. Giani T, Arena F, Vaggelli G, Conte V, Chiarelli A, Henrici De Angelis L, Fornaini R, Grazzini M, Niccolini F, Pecile P, et al. Large nosocomial outbreak of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* traced to clonal expansion of an mgrB deletion mutant. J Clin Microbiol. 2015;53(10):3341–4.
6. Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014;5:643.
7. Du H, Chen L, Tang Y, Kreiswirth BN. Emergence of the mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae. Lancet Infect Dis. 2016;16(3):287–8.
8. Yu H, Qu F, Shan B, Huang B, Jia W, Chen C, Li A, Miao M, Zhang X, Bao C, et al. Detection of mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae from different hospitals in China. Antimicrob Agents Chemother. 2016;60(8):5033–5.
9. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother. 2016;71(8):2066–70.
10. Haenni M, Poirel L, Kieffer N, Chatre P, Saras E, Metayer V, Dumoulin R, Nordmann P, Madec JY. Co-occurrence of extended spectrum beta lactamase and MCR-1 encoding genes on plasmids. Lancet Infect Dis. 2016;16(3):281–2.
11. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 10th ed. CLSI document M07-A10. Wayne: Clinical and Laboratory Standards Institute; 2015.
12. EUCAST. Breakpoints tables for interpretation of MICs and zone diameters, version 6.0; 2016.
13. He Q, Chen W, Huang L, Lin Q, Zhang J, Liu R, Li B. Performance evaluation of three automated identification systems in detecting carbapenem-resistant Enterobacteriaceae. Ann Clin Microbiol Antimicrob. 2016;15(1):40.

14. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int J Med Microbiol. 2005;295(6–7):383–404.
15. Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis. 2000;181(1):261–72.
16. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol. 2000;66(10):4555–8.
17. Johnson JR, Clermont O, Johnston B, Clabots C, Tchesnokova V, Sokurenko E, Junka AF, Maczynska B, Denamur E. Rapid and specific detection, molecular epidemiology, and experimental virulence of the O16 subgroup within *Escherichia coli* sequence type 131. J Clin Microbiol. 2014;52(5):1358–65.
18. Smith JL, Drum DJ, Dai Y, Kim JM, Sanchez S, Maurer JJ, Hofacre CL, Lee MD. Impact of antimicrobial usage on antimicrobial resistance in commensal *Escherichia coli* strains colonizing broiler chickens. Appl Environ Microbiol. 2007;73(5):1404–14.
19. Manges AR, Johnson JR, Foxman B, O'Bryan TT, Fullerton KE, Riley LW. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. N Engl J Med. 2001;345(14):1007–13.
20. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother. 2016;71:2066–70.
21. Li A, Yang Y, Miao M, Chavda KD, Mediavilla JR, Xie X, Tang YW. Complete sequences of mcr-1-harboring plasmids from extended-spectrum-beta-lactamase- and carbapenemase-producing Enterobacteriaceae. Antimicrob Agents Chemother. 2016;60(7):4351–4.
22. Yang RS, Feng Y, Lv XY, Duan JH, Chen J, Fang LX, Xia J, Liao XP, Sun J, Liu YH. Emergence of NDM-5 and MCR-1-producing *Escherichia coli* clone ST648 and ST156 from a single muscovy duck (*Cairina moschata*). Antimicrob Agents Chemother. 2016;60:6899–902.
23. Pitout JD. Extraintestinal pathogenic *Escherichia coli*: an update on antimicrobial resistance, laboratory diagnosis and treatment. Exp Rev Anti Infect Ther. 2012;10(10):1165–76.
24. Cortes-Cortes G, Lozano-Zarain P, Torres C, Castaneda M, Moreno Sanchez G, Alonso CA, Lopez-Pliego L, Gutierrez Mayen MG, Martinez-Laguna Y, Rocha-Gracia RD. Detection and molecular characterization of *Escherichia coli* strains producers of extended-spectrum and CMY-2 type beta-lactamases, isolated from turtles in Mexico. Vector Borne Zoonotic Dis. 2016;16:595–603.
25. Pan YS, Liu JH, Hu H, Zhao JF, Yuan L, Wu H, Wang LF, Hu GZ. Novel arrangement of the blaCTX-M-55 gene in an *Escherichia coli* isolate coproducing 16S rRNA methylase. J Basic Microbiol. 2013;53(11):928–33.
26. Schink AK, Kadlec K, Kaspar H, Mankertz J, Schwarz S. Analysis of extended-spectrum-beta-lactamase-producing *Escherichia coli isolates* collected in the GERM-Vet monitoring programme. J Antimicrob Chemother. 2013;68(8):1741–9.
27. Huang Y, Yu X, Xie M, Wang X, Liao K, Xue W, Chan EW, Zhang R, Chen S. Widespread dissemination of carbapenem-resistant *Escherichia coli* sequence type 167 strains harboring blaNDM-5 in clinical settings in China. Antimicrob Agents Chemother. 2016;60(7):4364–8.
28. Smith JL, Fratamico PM, Gunther NW. Extraintestinal pathogenic *Escherichia coli*. Foodborne Pathog Dis. 2007;4(2):134–63.
29. Lee S, Yu JK, Park K, Oh EJ, Kim SY, Park YJ. Phylogenetic groups and virulence factors in pathogenic and commensal strains of *Escherichia coli* and their association with blaCTX-M. Ann Clin Lab Sci. 2010;40(4):361–7.
30. Pitout JD, Laupland KB, Church DL, Menard ML, Johnson JR. Virulence factors of *Escherichia coli* isolates that produce CTX-M-type extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 2005;49(11):4667–70.
31. Skjot Rasmussen L, Ejrnaes K, Lundgren B, Hammerum AM, Frimodt Moller N. Virulence factors and phylogenetic grouping of *Escherichia coli* isolates from patients with bacteraemia of urinary tract origin relate to sex and hospital- vs. community-acquired origin. Int J Med Microbiol. 2012;302(3):129–34.
32. Vila J, Simon K, Ruiz J, Horcajada JP, Velasco M, Barranco M, et al. Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? J Infect Dis. 2002;186(7):1039–42.