Original Article

Detection of plasmid-mediated *qnr* genes among the quinolone-resistant *Escherichia coli* isolates in Iran

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Abstract

Introduction: Plasmid-mediated quinolone resistance, which complicates treatment, has been increasingly identified in *Escherichia coli* isolates worldwide. The purpose of this study was to identify the plasmid-mediated *qnrA* and *qnrB* genes among the quinolone-resistant *Escherichia coli* isolated from urinary tract infections in Iran.

Methodology: A total of 140 *Escherichia coli* isolates were collected between March and October 2012 from urinary tract infections in Khorram Abad, Iran. All isolates were tested for quinoloe resistance using the disk diffusion method. Also, all quinolone-resistant isolates were screened for the presence of the *qnrA* and *qnrB* genes by polymerase chain reaction. Minimum inhibitory concentrations (MICs) of ciprofloxacin for the *qnr*-positive isolates were determined.

Results: One hundred sixteen (82.8%) of 140 *Escherichia coli* isolates were nalidixic acid-resistant; among them, 14 (12.1%) and 9 (7.8%) were *qnrA* and *qnrB*-positive, respectively. Two quinolone-resistant isolates harbored both *qnrA* and *qnrB*. Among 63 ciprofloxacin-resistant isolates, 14 (22.2%) and 9 (14.3%) were found to carry *qnrA* and *qnrB* genes, respectively. The ciprofloxacin MIC range was 0.25–512 µg/mL for 23 *qnr*-positive *Escherichia coli* isolates, 18 of which had MICs values of 4–512 µg/mL.

Conclusion: Our study shows that the frequency of plasmid-mediated quinolone resistance genes among *E. coli* isolates in Iran is high.

Key words: quinolone resistance; *Escherichia coli*; *qnrA*; *qnrB*.

J Infect Dev Ctries 2014; 8(7):818-822. doi:10.3855/jidc.3746

(Received 29 April 2013 – Accepted 01 July 2013)

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Introduction

Escherichia coli (*E. coli*) is the major cause of urinary tract infections (UTIs), including cystitis and pyelonephritis [1]. Fluoroquinolones are the drugs of choice for the treatment of UTI infections in many countries due to widespread resistance to other antibiotics [1-3]. Based on its availability in oral and intravenous formulations, ciprofloxacin is preferred as the initial antibiotic for empiric therapy of UTIs in Iran [4]. Fluoroquinolone-resistant *E. coli* isolates have been increasingly reported worldwide, thus limiting therapeutic options [2,5].

Resistance to quinolones in *E. coli* isolates is usually associated with mutations in *gyrA* and *parC* genes [6,7]. In addition, efflux pumps and changes in the outer membrane proteins also contribute to chromosomal-mediated quinolone resistance [8]. Moreover, plasmid-mediated resistance genes play an important role in the development of low-level resistance to fluoroquinolones [9-11]. These genes include the *qnr* genes. The *qnrA* was the first plasmid-mediated gene to be detected, followed by *qnrB*, *qnrS*, *aac(6')-Ib*, and *qepA* genes that increase the MICs of quinolones four to eight times [12,13]. The *qnrB* is another plasmid-mediated quinolone resistant gene that has been identified worldwide and shares 43% amino acid identity with *qnrA* [13]. The horizontal transfer of plasmids carrying quinolone resistance determinants and the accumulation of chromosomal mutations play an important role in increasing rates of resistance [14,15].

Little is known about the frequency of *qnr* genes in *E. coli* isolates recovered from clinical specimens in Iran. Therefore, the aim of this study was to investigate the presence of plasmid-mediated *qnrA* and *qnrB* genes among the quinolone-resistant *E. coli* isolated from urinary tract infections of patients admitted to the two hospitals in Khorram Abad, Iran.

Methodology

Bacterial isolates and antimicrobial susceptibility testing

One hundred forty non-duplicate *Escherichia coli* isolates were collected from cases of urinary tract infections admitted to Shahid Madani and Shohaday-e-Ashayer hospitals in Khorram Abad, west Iran, between March and October 2012. All isolates were screened for quinolone and fluoroquinolone resistance using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. The following antibiotic disks were used for antimicrobial susceptibility testing: nalidixic acid (30 µg), norfloxacin (10 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), and ofloxacin (5 µg). The reference strain *E. coli* ATCC 25922 was used as a control. Results were interpreted as susceptible or resistant according to the criteria recommended by the CLSI and the manufacturer protocols (Mast Group, Bootle, UK).

MICs of ciprofloxacin for *E. coli* isolates that showed resistance to ciprofloxacin by the disk diffusion method, carrying *qnrA* or *qnrB* genes, were determined by the microbroth dilution method according to CLSI guidelines [16]. The quality control organisms were *E. coli* ATCC 25922 and *P. aeroginosa* ATCC 27853.

DNA extraction and qnr gene amplification

The DNA of 116 *E. coli* isolates found to be resistant to at least one antibiotic in the disk diffusion method were extracted using a DNA extraction kit (Bioneer, Daejeon, South Korea). Before DNA extraction, the antibiotic-resistant *E. coli* strains were cultured in Luria-Bertani broth at 37°C for 18 hours.

A polymerase chain reaction (PCR) assay was used to evaluate the presence of *qnrA* and *qnrB* resistance genes in quinolone- and fluoroquinolone-resistant strains of *E. coli*. The primers used for amplification of *qnrA* and *qnrB* genes were as follows: 5′-ATTTCTCACGCCAGGATTTG-3′ and 5′-GATCGGCAAAGGTTAGGTCA-3′ for *qnrA* determinant to detect a 516-bp amplicon, and 5'-GATCGTGAAAGCCAGAAAGG-3' and 5′ -ACGATGCCTGGTAGTTGTCC-3′ for *qnrB* determinant to detect a 469-bp amplicon [13]. The amplification conditions were 5 minutes at 95ºC and 35 cycles consisting of 94°C for 45 seconds, 51°C for 45 seconds and 72°C for 45 seconds, and 72°C for 7 minutes in the final extension. The electrophoresis of PCR products was performed on 1.2% agarose gel, and then the gels were stained in ethidium bromide for 15 minutes and visualized in a gel document system (Bio-Rad, Hemel Hempsted, UK).

Results

One hundred sixteen (82.8%) of 140 *E. coli* isolates were resistant to at least one of the tested antibiotics, and 24 (17.2%) were susceptible to nalidixic acid, ciprofloxacin, norfloxacin, levofloxacin, and ofloxacin. The results of antimicrobial susceptibility testing are shown in Table 1. Of 116 nalidixic acid-resistant isolates, 14 (12.1%) and 9 (7.8%) were positive for *qnrA* and *qnrB*, respectively, with 2 isolates co-harboring *qnrA* and *qnrB* genes (Figures 1 and 2). The frequency of *qnr* genes among 63 ciprofloxacin-resistant isolates was 14 (22.2%) for *qnrA* and 9 (14.3%) for *qnrB*.

The MICs for ciprofloxacin were 4–512 µg/mL in 11/14 isolates that showed ciprofloxacin resistance in the disk diffusion test and carried the *qnrA* determinants. Among the 14 ciprofloxacin-resistant *qnrA*-positive isolates, 8 (57.1%) showed MICs ≥ 64 µg/mL, and of the 9 ciprofloxacin-resistant *qnrB*-positive isolates, 7 (77.7%) had MICs in the range of 32–256 µg/mL. Six of the nine (66.7%) ciprofloxacin-resistant *qnrB*-positive isolates showed MICs ≥ 64 µg/ml (Table 2).

Table 1. Quinolone and fluoroquinolone-resistant isolates by disk diffusion method

Antibiotic	Isolates, n (%)	
	Sensitive	Resistant
Nalidixic acid	24 (17.2)	116 (82.8)
Ciprofloxacin	77 (55.0)	63 (45.0)
Norfloxacin	74 (52.9)	66 (47.1)
Ofloxacin	77 (55.0)	63 (45.0)
Levofloxacin	80 (57.1)	60 (42.9)

Figure 1. PCR amplification of *qnrA* gene in nalidixic acid-resistant *Escherichia coli* isolates. Electrophoresis of PCR product on 1.2% agarose gel. Lanes M, Marker 100 bp; lane 1, *E. coli qnrA*-positive control; lane 2, *E. coli qnrA*-negative control; lanes 3-16, *E. coli qnrA*- positive clinical isolates.

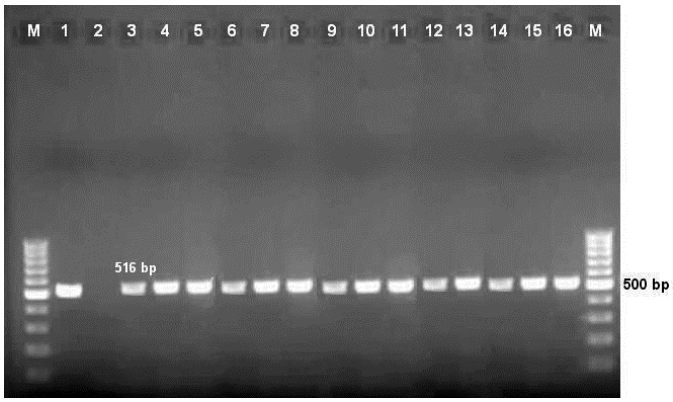

Figure 2. PCR amplification of *qnrB* gene in nalidixic acid-resistant *Escherichia coli* isolates. Electrophoresis of PCR product on 1.2% agarose gel. Lanes M, Marker 100 bp; lanes 1-9, *E. coli qnrB*-positive clinical isolates; lanes 10 and 11, *E. coli qnrB*-negative clinical isolates; lane 12, *E. coli qnrB*-positive control; lane 13 *E. coli qnrB*-negative control.

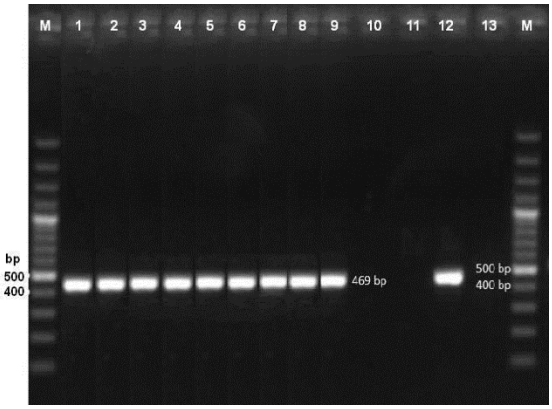

Table 2. Ciprofloxacin MICs values of *qnrA*- and *qnrB*-positive quinolone-resistant isolates

Quinolone resistant *qnr*-positive isolates, n (%)	Resistance gene	MIC of ciprofloxacin (µg/mL)	Total n (%)
1 (7.1)	*qnrA*	0.25	14 (100)
2 (14.3)	*qnrA*	1	
2 (14.3)	*qnrA*	4	
1 (7.1)	*qnrA*	8	
3 (21.5)	*qnrA*	64	
2 (14.3)	*qnrA*	128	
2 (14.3)	*qnrA*	256	
1 (7.1)	*qnrA*	512	
1 (11.1)	*qnrB*	0.5	9 (100)
1 (11.1)	*qnrB*	1	
1 (11.1)	*qnrB*	32	
2 (22.2)	*qnrB*	64	
3 (33.4)	*qnrB*	128	
1 (11.1)	*qnrB*	256	

Discussion

Plasmid-mediated quinolone resistance mechanisms play an important role in the development of quinolone and fluoroquinolone resistance [14,15]. Urinary tract infections caused by such resistant isolates can be difficult to treat [2,6]. In this study, the resistance rates to nalidixic acid and ciprofloxacin were 82.8% and 45%, respectively, indicating a high rate of resistance to quinolone and fluoroquinolone by *E. coli* isolates associated with UTIs in Khorram Abad. Since the development of nalidixic acid in 1962, the drug has been used for the treatment of UTIs for more than five decades [17]. The resistance rate for nalidixic acid, therefore, is expected to be higher than for fluoroquinolones. Ciprofloxacin is frequently used for treatment of UTIs in Iran [9]. The high ciprofloxacin resistance rate (45%) found in this study could be due to the inappropriate use of ciprofloxacin in Iran.

Other studies in most parts of the world have indicated that fluoroquinolone resistance in *E. coli* isolates is increasing [1,2,5,8,14]. In Pakistan, the frequencies of nalidixic acid and ciprofloxacin resistance among the *E. coli* isolated from UTIs were 84.2% and 36.5%, respectively, which is in agreement with our findings [18]. In addition, a study in China showed the frequency of ciprofloxacin resistance to have increased to 59.4% [19].

On the other hand, our findings are in contrast to studies conducted in the United States showing that

21% and 12% of uropathogenic *E. coli* were resistant to quinolones and fluoroquinolones, respectively [20]. There are many reasons for this discrepancy, including the situation of drug use in Iran where people take antimicrobial drugs without a prescription, differences in animal husbandry, and over–the-counter use of quinolones in veterinary medicine, as well as environmental conditions.

A significant relationship between fluoroquinolone use and resistance to these antibiotics has been documented [5]. The frequency of *qnr* genes in the ciprofloxacin-resistant *E. coli* isolates in the present study was higher than that found in China, where the rate of *qnr* genes among ciprofloxacin-resistant isolates of *E. coli* was 7.5% [19]. The results of a survey in Japan showed that *qnrA* was detected in 6.5% of *E. coli* clinical isolates and that the *qnrB* and *qnrS* genes were not found [21]. *QnrA* determinants were found in up to 32% of multidrug-resistant *Enterobacteriaceae* from blood cultures in Liverpool, United Kingdom [12]. In a similar study, approximately 30% of the quinolone-resistant *E. coli* isolates harbored the *qnr* genes [13]. Rates of 48% in Thailand and 86% in Vietnam have been reported [22,23]. In Greece, 10% of ciprofloxacin-resistant *E. coli* clinical isolates were *qnr*-positive [24], which is similar to the findings of our study.

In the current study, the findings that *qnrA* compared to *qnrB* was found in the majority of resistant isolates is similar to results from previous studies in Jamaica [14], the United Kingdom [12], and Spain [25], while in contrast to results of other studies [26,27]. For instance, in Korea, *qnrB* was the predominant *qnr* determinant identified among *E. coli* isolates [28]. Similarly, a remarkable dissemination of *qnrB* determinant in commensal enterobacteria isolates from healthy children was reported in Peru and Bolivia [29]. The results of a study in China revealed that *qnrA* was more common than the other three *qnr* gene groups [19], similar to our results. A high frequency of *qnr* determinants was identified in ciprofloxacin-resistant isolates compared to the nalidixic acid-resistant isolates, indicating that other mechanisms may contribute to nalidixic acid resistance.

The finding of our study that some *qnrA* and *qnrB*-negative isolates were also resistant to ciprofloxacin signifies that other *qnr* genes or resistance mechanisms, such as mutations in the target enzyme (*e.g.*, DNA gyrase and topoisomerase IV) and/or activation of efflux pumps, may be involved.

According to Jeong *et al*. (2005), the presence of a *qnr* determinant increases resistance to nalidixic acid and fluoroquinolones four to eight times [30]. In our study, the majority of ciprofloxacin-resistant isolates carrying *qnr* genes showed complete resistance to ciprofloxacin (MICs > 32 μg/mL). The high MIC values for *qnr*-positive ciprofloxacin-resistant isolates shows the extent of the therapeutic challenge for such resistant isolates.

In conclusion, our finding showed high frequencies of plasmid-mediated quinolone-resistant genes in urinary tract infection isolates of *E. coli* in Iran. Appropriate use of these antibiotics may be useful to limit the potential spread of resistant genes and reserve their application for therapeutic uses.

Acknowledgements

We wish to thanks Ms. Pegah Shakib and the staff of Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences for their help and technical assistance.

References

1. Kariuki S, Revathi G, Corkill J, Kiiru J, Mwituria J, Mirza N, Hart CA (2007) *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactamase. J Infect Dev Ctries 1: 257-262. doi:10.3855/jidc.361.
2. Karlowsky JA, Hoban DJ, de Corby MR, Laing NM, Zhanel GG (2006) Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from out patients are frequently multidrug-resistant: results from the North American urinary tract infection collaborative alliance-quinolone resistance study. Antimicrob Agents Chemother 50: 2251-2254.
3. Avgustin JA, Keber R, Zerjavic K, Orazem T, Grabnar M (2006) Emergence of the quinolone resistance-mediating gene *aac(6)-Ib-cr* in extended-spectrum lactamase-producing *Klebsiella* isolates collected in Slovenia between 2000 and 2005. Antimicrob Agents Chemother 51: 4171-4173.
4. Mojtahedzadeh M, Panahi Y, Fazeli MR, Najafi A, Pazouki M, Mahdi-Navehsi B, Bazzaz A, Naghizadeh MM, Beiraghdar F (2008) Intensive care unit-acquired urinary tract infections in patients admitted with sepsis: etiology, risk factors, and patterns of antimicrobial resistance. Int J Infect Dis 12: 312-318.
5. Goettsch W, van Pelt W, Nagelkerke N, Hendrix MG, Buiting AG, Petit PL, Sabbe LJ, van Griethuvsen AJ, Neelinga AJ (2000) Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands. J Antimicrob Chemother 46: 223-228.
6. Hooper DC **(**2001) Emerging mechanisms of fluoroquinolone resistance. Emerg Infect Dis 7: 337-341.
7. Mavroidi A, Miriagou V, Liakopoulos A, Tzelepi E, Stefos A, Dalekos GN, Petinaki A (2012) Ciprofloxacin-resistant *Escherichia coli* in Central Greece: mechanisms of resistance and molecular identification. BMC Infect Dis 12: 371.
8. Yamane K, Wachino J, Suzuki S, Arakawa Y (2008) Plasmid-mediated *qep*A gene among *Escherichia coli* clinical isolates from Japan. Antimicrob Agents Chemother 52: 1564-1566.

9. Pakzad I, Ghafourian S, Taherikalani M, Sadeghifard N, Abtahi H, Rahbar M, Mansory-Jamshidi N (2011) *Qnr* prevalence in extended spectrum beta-lactamases (ESBLs) and none-ESBLs producing *Escherichia coli* isolated from urinary tract infections in central of Iran. Iran J Bas Med Sci 14: 458-464.
10. Zhou TL, Chen XJ, Zhou MM, Zhao YJ, Luo HZ, Bao QU (2011) Prevalence of plasmid-mediated Quinolone Resistance in *Escherichia coli* isolated in Wenzhou, Southern China 2002-2008. Jpn J Infect Dis 64: 55-57.
11. Ruiz E, Saenz Y, Zarazaga M, Rocha-Gracia R, Martinez LM, Arlet G, Torres C (2012) *Qnr*, *aac(6')-Ib-cr* and *qep*A genes in *Escherichia coli* and *Klebsiella* spp.: genetic environments and plasmid and chromosomal location. J Antimicrob Chemother 67: 886-897.
12. Corkill JE, Anson JJ, Hart CA (2005) High prevalence of the plasmid-mediated quinolone resistance determinant *qnr*A in multidrug-resistant *Enterobacteriaceae* from blood cultures in Liverpool, UK. J Antimicrob Chemother 56: 1115-1117.
13. Stephenson S, Brown PD, Holness A, Wilks M (2010) The Emergence of *qnr*-mediated quinolone resistance among *Enterobacteriaceae* in Jamaica. West Indian Med J 59: 241.
14. Robicsek A, Sahm F, Strahilvitz J, Jacoby JA (2005) Broader distribution of plasmid-mediated quinolone resistance in United States. Antimicrob Agents Chemother 49: 3001-3003.
15. Bouchakour M, Zerouali KH, Claude JD, Amarouch H, Mdaghri NE, Courvalian P, Timinouni M (2010) Plasmid-mediated quinolone resistance in expanded spectrum beta lactamase producing *entrobacteriaceae* in Morocco. J Infect Dev Ctries 4: 799-803. doi:10.3855/jidc.796.
16. Clinical and Laboratory Standards Institute (2011) Performance Standards for Antimicrobial Susceptibility Testing. Twenty-First Informational Supplement. Document M100-S21. CLSI: Wayne, PA.
17. Emmerson AM, Jones AM (2003) The quinolones: decades of development and use. J Antimicrob Chemother 51: 13-20.
18. Muhammad I, Uzma M, Yasmin B, Mehmood Q, Habib B (2011) Prevalence of antimicrobial resistance and integrons in *Escherichia coli* from Punjab, Pakistan Bra J Microbiol 42: 462-466.
19. Wang A, Yang Y, Lu Q, Wang Y, Chen Y, Deng L, Ding H, Deng Q, Zhang H, Wang C, Liu L, Xu X, Wang L, Shen X (2008) Presence of *qnr* gene in *Escherichia coli* and *Klebsiella pneumonia* resistant to ciprofloxacin isolated from pediatric patients in China. BMC Infect Dis 8: 1-6.
20. Moreno E, Prats G, Sabate M, Perez T, Johnson JR, Andreu A (2006) Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. Antimicrob Agents Chemother 57: 204-211.
21. Odea T, Saitob R, Kumitaa W, Satoa K, Okugawab S, Moriyab K, Koikeb K, Okamura N (2009) Analysis of plasmid-mediated multidrug resistance in *Escherichia coli* and *Klebsiella oxytoca* isolates from clinical specimens in Japan. Int J Antimicrob Agents 34: 347-350.
22. Poirel L, Rodriguez-Martinez JM, Mammeri H, Liard A, Nordmann P (2005) Origin of plasmid-mediated quinolone resistance determinant *Qnr*A. Antimicrob Agents Chemother 49: 3523-3525.
23. Le TM, Baker S, Le TP, Le TP, Cao TT, Tran TT, Nguyen VM, Campbell JI, Lam MY, Nguyen TH, Nguyen VV, Farrar J, Schultsz C (2009) High prevalence of plasmid-mediated quinolone resistance determinants in commensal members of the *Enterobacteriaceae* in Ho Chi Minh City, Vietnam. J Med Microbiol 58: 1585-1592.
24. Vasilaki O, Ntokou E, Ikonomidis A, Sofianou D, Frantzidou F, Alexiou-Daniel S, Maniatis AN, Pournaras S (2008) Emergence of the plasmid-mediated quinolone resistance gene *qnrS1* in *Escherichia coli* isolates in Greece. Antimicrob Agents Chemother 52: 2996-2997.
25. Lavilla S, González-López JJ, Sabaté M, García-Fernández A, Larrosa MN, Bartolomé RM, Carattoli A, Prats G (2008) Prevalence of *qnr* genes among extended spectrum β-lactamase-producing enterobacterial isolates in Barcelona Spain. J Antimicrob Chemother 612: 291-295.
26. Wullt B, Bergsten G, Samuelsson L, Svanborg C (2002) The role of P fimbriae for *Escherichia coli* establishment and mucosal inflammation in the human urinary tract. Int J Antimicrob Agents 19: 522-538.
27. Bergsten G, Wullt B, Svanborg C (2005) *Escherichia coli* fimbriae bacterial persistence and host response induction in the human urinary tract. Int J Med Microbiol 295: 487-502.
28. Kim MH, Lee HJ, Park KS, Suh JT (2010) Molecular characteristics of extended spectrum beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* and the prevalence of qnr in extended spectrum beta-lactamase isolates in a tertiary care hospital in Korea. Yonsei Med J 51: 768-774.
29. Pallecchi L, Riccobono E, Mantella A, Bartalesi F, Sennati S, Gamboa H, Gotuzzo E, Bartoloni A, Rossolini GM (2009) High prevalence of *qnr* genes in commensal enterobacteria from healthy children in Peru and Bolivia. Antimicrob Agents Chemother 56: 2632-2635.
30. Jeong JY, Yoon HJ, Kim ES, Lee Y, Choi SH, Kim NJ, Woo JH, Kim YS (2005) Detection of *qnr* in clinical isolates of *Escherichia coli* from Korea. Antimicrob Agents Chemother 49: 2522-2524.

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Conflict of interests: No conflict of interests is declared.