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Expression of the Fluoroquinolones Efflux Pump Genes *acr*A and *mdf*A in Urinary *Escherichia coli* Isolates

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Abstract

Escherichia coli is one of the most frequent causes of urinary tract infections. Efflux system overexpression is reported to contribute to *E. coli* resistance to several antibiotics. Our aim in this study was to investigate the relation between antibiotic resistance and the expression of the efflux pump genes *acr*A and *mdf*A in *E. coli* by real-time reverse transcription-PCR. We tested the *in vitro* susceptibilities to 12 antibiotics in 28 clinical isolates of *E. coli* obtained from urine samples. We also determined the minimum inhibitory concentrations of levofloxacin to these samples. We then revealed significant correlations between the overexpression of both *mdf*A and *acr*A and MICs of levofloxacin. In conclusion, we demonstrated that the increased expression of efflux pump genes such as *mdf*A and *acr*A can lead to levofloxacin resistance in *E. coli*. These findings contribute to further understanding of the molecular mechanisms of efflux pump systems and how they contribute to antibiotic resistance.

Key words: *Escherichia coli*, *acr*A and *mdf*A genes, efflux pump, levofloxacin, overexpression

Introduction

Antimicrobial abuse is one of the major factors contributing to the development and maintenance of antimicrobial resistance in bacteria. The issue is of utmost importance in many developing countries, where the sale of antimicrobials is often unrestricted (Okeke *et al*., 2005).

In Egypt, *Escherichia coli* is among the most common pathogens isolated from patients with urinary tract infections and it has a high prevalence of resistance to antibiotics (Shaheen *et al*., 2004; Mohamed Al-Agamy *et al*., 2006).

Bacterial resistance to antimicrobial agents is a threat to public health in Egypt. The consequences of resistance affect not only the ability to treat the infection, but also the cost and duration of treatment (Shaheen *et al*., 2004; Mohamed Al-Agamy *et al*., 2006).

Fluoroquinolone resistance is on the rise. The emergence of this resistance in nearly all species of bacteria was documented soon after the introduction of these compounds for clinical use (Acar and Goldstein, 1997).

Levofloxacin resistance has been shown to increase more than 5-fold in 6 years. Rapid emergence of resistance may have been fuelled by the increase in the rate of levofloxacin prescribing (Johnson *et al*., 2008). A study showed that the annual rates of levofloxacin resistance of *E. coli* were 29.49% in 2005, 26.51% in 2006, 40.21% in 2007, 43.20% in 2008, and 31.75% in 2009 (Jang *et al*., 2011). The persistent increase in fluoroquinolone resistance rates influences patient management and demands a change in some existing guidelines for the treatment (Paterson, 2004; Wagenlehner *et al*., 2011; Han *et al*., 2012).

The overexpression of efflux pumps contributes to multidrug resistance (MDR) in *E. coli.* Almost all Gram-negative bacteria have genes for efflux pumps. Two transporters, AcrAB and MdfA, which belong to two distinct families of efflux pumps, have the ability to efflux quinolones in *E. coli*. Most of these efflux genes are either underexpressed or not expressed at all. When they are overexpressed, they are associated with MDR in *E. coli* (Yang, 2003; Yasufuku *et al*., 2011).

The AcrAB-TolC system of *E. coli* is among the better characterized resistance-nodulation-cell division superfamily (RND) systems. In addition to recognizing many fluoroquinolones, this system exports a diversity of agents including tetracycline, β-lactams, chloramphenicol, erythromycin, rifampicin, disinfectants, dyes, and organic solvents. AcrA is a lipoprotein situated in

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the periplasm; the inner membrane protein, AcrB, is a proton-motive-force transporter of the RND type. Strains lacking in the AcrAB proteins are hypersusceptible to many quinolones; on the other hand, fluoroquinolone-resistant efflux mutants frequently overproduce the periplasmic protein AcrA. Such mutants display decreased susceptibility to ciprofloxacin, tetracycline, ampicillin, and chloramphenicol. Removal or inactivation of proteins that suppress expression of AcrAB (AcrR and AcrS) reduces fluoroquinolone susceptibility (Drlica *et al*., 2012).

MdfA is a paradigm of multidrug-resistance antiporters. It is a member of the major facilitator superfamily (MFS) and is a transmembrane-spanning integral inner membrane protein (Edgar and Bibi, 1997).

In laboratory-generated mutants, overexpression of the efflux pumps AcrAB or MdfA have been shown to cause three to six fold increase in fluoroquinolone resistance (Yang, 2003). The simultaneous overexpression of AcrAB and MdfA results in synergistic increases in resistance to fluoroquinolones (Yang, 2003).

The overexpression of AcrA and MdfA in quinolone resistance in *E. coli* has been thoroughly investigated, yet data about it in Egypt, up until the date this paper has been written, has been really scarce.

Our aim in this study was to investigate the relation between antibiotic resistance and the expression of the efflux pump genes in *E. coli* by real-time quantitative polymerase chain reaction (RT-qPCR).

Experimental

Material and Methods

***E. coli* strains and growth conditions.** A total of 28 non-duplicate *E. coli* isolates were used in this study obtained from patients with urinary tract infection (UTI) treated in Damanhour General Hospital, Damanhour, Egypt between the years 2013 and 2014. Post treatment isolates were excluded from this study. The isolates were identified with conventional biochemical tests. All isolates were deemed to be clinically significant ($>10^5$ CFU/ml). They were selected according to their sensitivity to levofloxacin in order to correlate their sensitivity profile with the overexpression of efflux pump genes. *E. coli* isolates with intermediate susceptibility were not classified as being resistant. The strains were propagated at 37°C in Luria-Bertani (LB) broth.

Susceptibility testing. The following 12 antimicrobial discs were selected according to CLSI guidelines (CLSI, 2015): amoxicillin (AML), amoxicillin-clavulanic acid (AMC), cefuroxim (CMX), cefalexin (CL), cefepime (FEP), ceftriaxone (CRO), chloramphenicol (C), gentamycin (CN) tetracycline (TE), levofloxacin (LVX), meropenem (MEM), azithromycin (AZM) (Oxoid Ltd., Basingstoke, United Kingdom) on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, United Kingdom). Zone sizes were measured to the nearest millimetre. Using the published CLSI guidelines, the susceptibility or resistance of the organism to each drug tested were then determined. *E. coli* ATCC 25922 (LGC Standards GmbH, Wesel, Germany) was used as a control (CLSI, 2015).

Determination of the minimum inhibitory concentration (MIC) to levofloxacin. Determination of the MIC of levofloxacin was done by using the two-fold dilution method according to CLSI guidelines (CLSI, 2015). MICs were performed in 96-well microplates (Greiner, Wemmel, Belgium). Briefly, serial two fold dilutions of 2x strength antibiotic were performed in sterile distilled water. An overnight culture of the tested isolate in Mueller-Hinton Broth (MHB) was diluted in fresh double strength MHB till it reaches the concentration of 10^5 CFU/ml.

Fifty µl suspension of the organism in double strength MHB was added to the well with 0.05 ml of the antibiotic solution, and the microtiter plates were incubated aerobically at 35°C. The MIC was then defined as the lowest concentration of the antibiotic in which there is no visible growth after overnight incubation. Results were recorded in µg/ml after incubation at 35°C for 18 hours (CLSI breakpoints for levofloxacin are ≤ 2 µg/ml for susceptibility and ≥ 8 µg/ml for resistance).

RNA extraction and quantification of RNA expression using RT-qPCR (Taylor *et al*., 2010). All *E. coli* isolates were cultured at 37°C for 14 h on MacConkey agar plates (Oxoid Ltd., Basingstoke, United Kingdom), then cultured in 4 ml of Luria-Bertani medium (Oxoid Ltd., Basingstoke, United Kingdom) for 8 h. Total RNA was obtained from mid-logarithmic growth-phase bacterial cultures with Isolate II RNA Micro Kit (Bioline, Michigan, USA) (Pfaffl *et al*., 2002; Keeney *et al*., 2008). Genomic DNA contamination was removed by an on-column RNase-free DNase I digestion. RNA purity was assessed spectrophotometrically by measuring the $OD_{260/280}$ ratio by NanoDrop™ 8000 Spectrophotometer (Thermo Fisher Scientific – Delaware, USA). RNA integrity was assessed by visualization of one large and one small band of RNA after electrophoresis (Fleige and Pfaffl, 2006).

The quantification protocol was performed immediately following the quality control assessment. The *acr*A and *mdf*A genes were quantified by RT-qPCR using a SensiFAST™ SYBR No-ROX One-Step Kit (2x) (Bioline, United Kingdom) in a StepOne™ Real-Time PCR System (StepOne™ system, Applied biosystems, USA). The reaction mixture was prepared in a total volume of 20 µl dispensed in MicroAmp® optical microplates

Table I
Primers used in this study

Gene	Primer (5'-3')	Amplicon length (bp)	Reference
*mdf*A F *mdf*A R	CGGCAACGATATGATTCAAC CAGTGACAGTTTCTCGCCTA	523	Yasufuku *et al.*, 2011
*acr*A F *acr*A R	CTCTCAGGCAGCTTAGCCCTAA TGCAGAGGTTCAGTTTTGACTGTT	107	Swick *et al.*, 2011
*gap*A F *gap*A R	ACTTCGACAAATATGCTGGC CGGGATGATGTTCTGGGAA	215	Yasufuku *et al.*, 2011

(ThermoFisher Scientific, Michigan, USA). Each reaction mixture contained 0.5 µM of both forward and reverse primers, 10 µl of SensiFAST™ SYBR No-ROX One-Step Kit (2x) mix, 0.2 µl of reverse transcriptase, 0.4 µl of Ribosafe RNA inhibitor, 4 µl of template RNA, and nuclease-free water to complete the volume. The oligonucleotide primers for the amplification were adapted from prior studies and are listed in (Table I) (Swick *et al*., 2011; Yasufuku *et al*., 2011).

PCR-grade water was used as a negative control. Genomic DNA from the *E. coli* ATCC 25922 strain was the positive control. The melting curve analysis ensured that only a single PCR product was amplified. A no-RT control was included by omitting the reverse transcriptase from the reaction.

Each sample was placed in triplicate on a 48-well plate and subjected to one-step reverse transcription at 50°C for 30 min, 40 cycles of denaturation at 94°C for 15 s, annealing at 50°C for 30 s and extension at 72°C for 30 s. The *Cq* means of almost all samples in our study were less than 30 (Yasufuku *et al*., 2011).

Using the Applied biosystems stepone™ system software the relative quantification of the expression of the target genes was calculated relative to *gap*A, a housekeeping gene, and then the expression rate of each efflux pump gene was determined by using *E. coli (*ATCC 25922) as the control (Pfaffl *et al*., 2002; Bohnert *et al*., 2007).

Correlation of overexpression of efflux pump genes with MICs of each antibiotic. The overexpression of efflux pump genes was identified as ≥ 2-fold gene expression compared to that of the standard strain *E. coli* (ATCC 25922) in qRT-PCR (Keeney *et al*., 2008). The correlation of the overexpression of efflux pump genes with the MICs of levofloxacin was then determined. The correlations between the pattern of resistance and the expression of both genes together with the MICs of levofloxacin have also been determined.

Statistical analysis. Statistical analyses were performed by using Statistical Package for Social Sciences (SPSS) software version 17 with Spearman's correlation test; $p < 0.05$ was considered statistically significant.

Results

Susceptibility testing. Susceptibility testing for the 12 antibiotics was performed according to CLSI guidelines (CLSI, 2015). It showed that 79% of the isolates were MDR. MDR is most commonly defined as resistance to ≥ 3 classes of antibiotics (Magiorakos *et al*., 2012). The resistance patterns of the isolates (the number of antibiotics to which each isolate is resistant) to the studied antibiotics are shown in (Table II).

Table II
Susceptibility of antimicrobial agents

Antibiotic	No. (%) of isolates (n = 28)		
	Susceptible	Intermediate	Resistant
AML	0 (0)	1 (3.6)	27 (96.4)
AMC	10 (35.7)	6 (21.4)	12 (42.8)
CMX	6 (21.4)	4 (14.3)	18 (64.3)
CL	6 (21.4)	2 (7.1)	20 (71.4)
FEP	14 (50)	0 (0)	14 (50)
CRO	10 (35.7)	2 (7.1)	16 (57.1)
C	20 (71.4)	0 (0)	8 (28.6)
CN	14 (50)	0 (0)	14 (50)
TE	10 (35.7)	0 (0)	18 (64.3)
LEV	0 (0)	3 (10.7)	25 (89.3)
MEM	28 (100)	0 (0)	0 (0)
AZM	8 (28.6)	0 (0)	20 (71.4)

Minimum Inhibitory Concentration (MIC) and gene expression. Determination of the MIC of levofloxacin was done by using the two-fold dilution method according to CLSI guidelines (Clinical and Laboratory Standards Institute. 3rd ed. 2015). All the tested isolates (100%) were levofloxacin resistant. Results of determination of MIC of levofloxacin are shown in (Table III).

Expression of *acr*A and *mdf*A genes. Among the 28 isolates, 23 isolates (82.1) % showed overexpression of *mdf*A ranging from 2–34.47 folds, and 22 (78.6%) showed overexpression of *acr*A ranging from 2–9.52 folds (Table III).

Table III
Resistance pattern of strains, MIC of levofloxacin and analysis of quantification of RNA expression using qRT-PCR

Sample no.	Resistance pattern	MIC of Levofloxacin (μg/ml)	Expression of	
			*mdf*A*	*acr*A*
1	8	32	2.52	2.66
2	11	128	4.93	7.71
3	11	32	2.49	0.43
4	4	64	4.90	3.93
5	10	128	11.95	9.18
6	2	8	1.15	1.45
7	6	8	1.03	0.89
8	10	64	2.83	6.44
9	9	64	3.47	9.52
10	4	32	1.70	8.18
11	10	256	34.47	2.55
12	9	256	11.45	15.1
13	6	128	4.07	8.65
14	8	32	2.6	3.05
15	11	64	3.96	2.87
16	3	32	1.78	2.43
17	9	128	7.76	7.98
18	11	64	2.94	3.32
19	8	256	5.58	6.93
20	10	32	2.67	3.32
21	2	8	1.06	0.95
22	10	128	7.93	7.39
23	4	32	2.55	1.89
24	9	64	4.32	3.99
25	6	32	2.78	2.09
26	10	32	3.65	1.24
27	8	128	6.44	7.84
28	6	64	3.99	2.33

* Average of three independent replicates

Correlation of overexpression with MIC and patterns of antibiotic resistance. The overexpression of efflux pump genes was defined as ≥ 2-fold gene expression compared to that of the standard strain *E. coli* (ATCC 25922) in qRT-PCR. A strong positive correlation of the overexpression of both of *mdf*A and *acr*A with MICs of levofloxacin was detected (Spearman correlation 0.93 and 0.72 respectively, $P < 0.05$) by bivariate analysis.

Moderate positive correlation was found between the expression of *mdf*A and the pattern of antimicrobial resistance of each isolate (Spearman correlation 0.45, $P < 0.05$). This was not the case with the expression of *acr*A where no significant correlation with the pattern of antimicrobial resistance was found.

Moderate positive correlation was also found between levofloxacin MIC and the pattern of antimicrobial resistance of each isolate (Spearman correlation 0.45, $P < 0.05$)

Discussion

Multidrug resistance is a growing public health concern worldwide (Okeke *et al.*, 2005). The increasing incidence of resistance among pathogenic *E. coli* makes the treatment difficult with an unpredictable outcome (Kariuki *et al.*, 2007). Resistance to quinolones has reached 50% in some parts of the world (Kronvall, 2010). This leads to a challenge in the use of this class of drugs. It is now necessary to investigate the possible molecular mechanisms by which *E. coli* strains acquire antibiotic resistance in an attempt to prevent further spread of these kinds of resistant strains and prevent the occurrence of new resistant ones.

In the present study we examined 28 *E. coli* isolates, all isolates (100%) were levofloxacin resistant and 79% were MDR. Similar to our results, Swick *et al.* (2011) found that no isolate categorized as MDR was flouroquinolone susceptible. They also suggested the possibility of an underlying correlation between fluoroquinolone resistance and MDR, this was confirmed in our study where moderate positive correlation was found between levofloxacin MIC and the pattern of antimicrobial resistance of each isolate (Spearman correlation 0.45, $P < 0.05$)

There is a widespread, escalating use of fluoroquinolones. Even contrary to guidelines that recommend them in only select situations, the frequent use of fluoroquinolone antibiotics has been observed in nearly all settings. Quinolone antibiotics now have exceeded sulfa antibiotics in recent years as the most commonly prescribed treatment for outpatient UTIs in women (Kallen *et al.*, 2006).

We quantified the expression of the efflux pump genes known to affect fluoroquinolone resistance in a single quantitative study and we used *gap*A as a house keeping gene. Housekeeping genes provided internal controls, which were used as a measure of mRNA expression levels irrespective of growth condition (Szabo *et al.*, 2004). Jandu *et al.* (2009) reported that the expression of *gap*A was similar when detected under four different growth conditions so it was chosen as an internal control for our study.

We found that most of the levofloxacin resistant isolates overexpressed both *mdf*A and *acr*A genes (82.1% and 78.6% respectively). This was with agreement of other studies, where overexpression of *acr*A together with *acr*B led to a three- to six-fold increase in fluoroquinolone resistance, while augmenting the level of *acr*AB through the overexpression of the tran-

scriptional regulator, *sdi*A, resulted in about the same (approximately six-fold) increase in drug resistance. Yasufuku *et al*. (2011) revealed a significant correlation of the overexpression of *mdf*A with higher MICs of levofloxacin (Rahmati *et al.*, 2002; Yang, 2003).

In general the more severe the MDR phenotype, the higher the likelihood that the isolate also overexpressed *acr*AB (Nikaido and Pagès, 2012). Han *et al*. (2012) found that out of 89 patients with fluoroquinolone resistant isolates, 49.4% overexpressed *acr*AB.

A synergy in quinolone resistance was shown when *acr*AB was overexpressed simultaneously with *nor*E or *mdf*A. Deletion of *acr*AB alone and all of the three pumps together had the same effect on the susceptibility of fluoroquinolones. The authors also found that the strain with *acr*AB deletion were the most susceptible when compared to mutants strains with deletion in *nor*E and/or *mdf*A. The maximum quinolone resistance mediated by efflux pumps was suggested to be ~10-fold, irrespective of any increase in production of these pumps (Yang, 2003).

Studies conducted with efflux pump deleted (ΔAcrAB) strain delayed the emergence of resistance suggesting that the inhibition of AcrAB efflux pump could be a strong strategy for slowing the development of resistance in clinically important Gram-negative bacteria (Singh *et al*., 2012)

In our study 17.9% and 21.4% of the levofloxacin resistant isolates didn't show over expression of *mdf*A and *acr*A respectively, thus flouroquinolone resistance may be contributed to other mechanisms such as gyrase or topoisomerase IV mutations. Morgan-Linnell *et al*. (2009) reported mutations in gyrase and/topoisomerase IV in 100% of the high-level-resistant *E. coli* clinical isolates, while over production of the efflux pump (AcrA) contributed approximately 33% to the overall quinolone resistance. As to the *mdf*A gene, one of the levofloxacin susceptible isolates showed very slight overexpression.

It should be noted that expression of pump genes can be induced by natural substrates encountered during infection. For example, indole and bile, which would be encountered in the mammalian gut, induce expression of *acr*AB in *E. coli* and *Salmonella* (Sun *et al*., 2014).

Though increased efflux pump expression correlates with increase in levofloxacin MIC, we did not compare expression of efflux pumps in levofloxacin sensitive strains. Thus, this has to be investigated as efflux pump expression might be high in these strains too if they are resistant to other antibiotics since both pumps are broad spectrum. Some studies have found that resistance to antibiotics through the same efflux pump is common (Poole, 2005).

An approach of blocking the efflux pump protein or gene has been studied. This falls under the class of biological inhibition of efflux pumps. The efflux pumps could be switched off with the means of specific antibodies (Yoshihara and Inoko, 2011). The genes encoding these pumps or their regulators could be blocked by means of the antisense strategies. The antisense approach has been revealed to work for AcrAB efflux pump in *E. coli* and has also been patented (Oethinger and Levy, 2001; 2011).

Efflux pump inhibitors can also be used for the evaluation of the effect of efflux pumps on resistance emergence, however there are two major concerns with such inhibitors. First, some of these inhibitors have been found to be substrates of efflux pumps themselves, thus questioning their credibility (Lomovskaya and Bostian, 2006). Second, the concentrations required for efflux pump inhibitory activity are often associated with toxicity and hence, are not clinically relevant (Schmitz *et al*., 1998).

While, only one Gram-negative bacterial species has been evaluated in our study, it is imperative to investigate other bacterial species to increase the clinical relevance of our results. Different inhibitory techniques can be utilized to study the different efflux pumps. These should not only be effective against multiple efflux pumps in one bacterial species but also against efflux pumps in more than one bacterial species.

In conclusion, we demonstrated a significant positive correlation between the overexpression of AcrA and MdfA efflux pumps and the resistance to levofloxacin in *E. coli* clinical isolates. These findings contribute to further understanding of the molecular mechanisms of efflux pump systems and how they contribute to antibiotic resistance and suggest that developing new EPI could lead to treatments for flouroquinolones resistant bacteria.

Resistance emergence presents a debilitating challenge in the management of infectious diseases. Data from this study might add to our understanding of resistance development. Our findings suggest that the inhibition of efflux pumps could be a potential strategy to thwart the problem of antibiotic resistance. Considering the genetic plasticity in the bacteria, these findings just seem to be the tip of the iceberg and much needs to be unravelled in the future.

Literature

Acar J.F. and F.W. Goldstein. 1997. Trends in bacterial resistance to fluoroquinolones. *Clin. Infect. Dis.* 24(Suppl 1): S67–S73.

Bohnert J.A., S. Schuster, E. Fahnrich, R. Trittler, and W.V. Kern. 2007. Altered spectrum of multidrug resistance associated with a single point mutation in the *Escherichia coli* RND type MDR efflux pump YhiV (MdtF). *J. Antimicrob. Chemother.* 59: 1216–1222.

Clinical and Laboratory Standards Institute (CLSI). 2015. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 3rd ed. CLSI document M31-A3. Clinical and Laboratory Standards.

Drlica K., X. Zhao, M. Malik and T.K.R. Salz. 2012. Fluoroquinolone resistance: mechanisms, restrictive dosing, and anti-mutant screening strategies for new compounds, pp. 485–514. In: Dougherty T.J. and M.J. Pucci (eds). *Antibiotic Discovery and Development*. 1st ed. Springer, US.

Edgar R. and E. Bibi. 1997. MdfA, an *Escherichia coli* multidrug resistance protein with an extraordinarily broad spectrum of drug recognition. *J. Bacteriol.* 179: 2274–2280.

Fleige S. and M.W. Pfaffl. 2006. RNA integrity and the effect on the real-time qRT-PCR performance. *Mol. Aspects Med.* 27: 126–139.

Han J.H., I. Nachamkin, P. Tolomeo, X. Mao, W.B. Bilker and E. Lautenbach. 2012. Risk factors for efflux pump overexpression in fluoroquinolone-resistant *Escherichia coli*. *J. Infect. Dis.* 206: 1597–1603.

Jandu N., N.K.L. Ho, K.A. Donato, M.A. Karmali, M. Mascarenhas, S.P. Duffy, C. Tailor and P.M. Sherman. 2009. Enterohemorrhagic *Escherichia coli* O157:H7 gene expression profiling in response to growth in the presence of host epithelia. *PLoS One* 4: e4889.

Jang W.H., D.H. Yoo and S.W. Park. 2011. Prevalence of and risk factors for levofloxacin-resistant *E. coli* isolated from outpatients with urinary tract infection. *Korean J. Urol.* 52: 554–559.

Johnson L., A. Sabel, W.J. Burman, R.M. Everhart, M. Rome, T.D. MacKenzie, J. Rozwadowski, P.S. Mehler and C.S. Price. 2008. Emergence of fluoroquinolone resistance in outpatient urinary *Escherichia coli* isolates. *Am. J. Med.* 121: 876–884.

Kallen A.J., H.G. Welch and B.E. Sirovich. 2006. Current antibiotic therapy for isolated urinary tract infections in women. *Arch. Intern. Med.* 166: 635–639.

Kariuki S., G. Revathi, J. Corkill, J. Kiiru, J. Mwituria, N. Mirza and C.A. Hart. 2007. *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams. *J. Infect. Dev. Ctries.* 1: 257–262.

Keeney D., A. Ruzin, F. McAleese, E. Murphy and P.A. Bradford. 2008. MarA-mediated overexpression of the AcrAB efflux pump results in decreased susceptibility to tigecycline in *Escherichia coli*. *J. Antimicrob. Chemother.* 61: 46–53

Kronvall G. 2010. Antimicrobial resistance 1979–2009 at Karolinska hospital, Sweden: normalized resistance interpretation during a 30-year follow-up on *Staphylococcus aureus* and *Escherichia coli* resistance development. *APMIS* 118: 621–639.

Lomovskaya O. and K.A. Bostian. 2006. Practical applications and feasibility of efflux pump inhibitors in the clinic – a vision for applied use. *Biochem. Pharmacol.* 71: 910–918.

Magiorakos A.P., A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske, S. Harbarth, J.F. Hindler, G. Kahlmeter and others. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* 18: 268–281.

Mohamed Al-Agamy M.H., M.S. El-Din Ashour and I. Wiegand. 2006. First description of *CTX-M* beta-lactamase-producing clinical *Escherichia coli* isolates from Egypt. *Int. J. Antimicrob. Agents* 27: 545–548.

Morgan-Linnell S.K., L. Becnel Boyd, D. Steffen and L. Zechiedrich. 2009. Mechanisms accounting for fluoroquinolone resistance in *Escherichia coli* clinical isolates. *Antimicrob. Agents Chemother.* 53: 235–241.

Nikaido H. and J.M. Pagès. 2012. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. *FEMS Microbiol. Rev.* 36: 340–363.

Oethinger M. and S.B. Levy. 2001. Methods of reducing microbial resistance to drugs. Retrieved from http://www.google.com/patents/CA2378883A1?cl=en, 2016.02.10.

Oethinger M. and S.B. Levy. 2011. Methods of screening for compounds that reduce microbial resistance to fluoroquinolones. Retrieved from https://www.google.com.ar/patents/US8012711, 2016.02.10.

Okeke I.N., R. Laxminarayan, Z. a Bhutta, A.G. Duse, P. Jenkins, T.F. O'Brien, A. Pablos-Mendez and K.P. Klugman. 2005. Antimicrobial resistance in developing countries. Part I: Recent trends and current status. *Lancet Infect. Dis.* 5: 481–493.

Paterson D.L. 2004. "Collateral damage" from cephalosporin or quinolone antibiotic therapy. *Clin. Infect. Dis.* 38(Suppl 4):S341–S345.

Pfaffl M.W., G.W. Horgan and L. Dempfle. 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res.* 30: e36

Poole K. 2005. Efflux-mediated antimicrobial resistance. *J. Antimicrob. Chemother.* 56: 20–51

Rahmati S., S. Yang, A.L. Davidson and E.L. Zechiedrich. 2002. Control of the *AcrAB* multidrug efflux pump by quorum-sensing regulator *SdiA*. *Mol. Microbiol.* 43: 677–685.

Schmitz F.J., A.C. Fluit, M. Lückefahr, B. Engler, B. Hofmann, J. Verhoef, H.P. Heinz, U. Hadding and M.E. Jones. 1998. The effect of reserpine, an inhibitor of multidrug efflux pumps, on the *in vitro* activities of ciprofloxacin, sparfloxacin and moxifloxacin against clinical isolates of *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 42: 807–810.

Shaheen H.I., S.B. Khalil, M.R. Rao, R. Abu Elyazeed, T.F. Wierzba, L.F. Peruski Jr., S. Putnam, A. Navarro, B.Z. Morsy and others. 2004. Phenotypic profiles of enterotoxigenic *Escherichia coli* associated with early childhood diarrhea in rural Egypt. *J. Clin. Microbiol.* 42: 5588–5595.

Singh R., M.C. Swick, K.R. Ledesma, Z. Yang, M. Hu, L. Zechiedrich and V.H. Tam. 2012. Temporal interplay between efflux pumps and target mutations in development of antibiotic resistance in *Escherichia coli*. *Antimicrob. Agents Chemother.* 56: 1680–1685.

Sun J., Z. Deng and A. Yan. 2014. Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations. *Biochem. Biophys. Res. Commun.* 453: 254–267.

Swick M.C., S.K. Morgan-Linnell, K.M. Carlson and L. Zechiedrich. 2011. Expression of multidrug efflux pump genes *acr*AB-*tol*C, *mdf*A, and *nor*E in *Escherichia coli* clinical isolates as a function of fluoroquinolone and multidrug resistance. *Antimicrob. Agents Chemother.* 55: 921–924.

Szabo A., C.M. Perou, M. Karaca, L. Perreard, R. Palais, J.F. Quackenbush and P.S. Bernard. 2004. Statistical modeling for selecting housekeeper genes. *Genome Biol.* 5: R59.

Taylor S., M. Wakem, G. Dijkman, M. Alsarraj and M. Nguyen. 2010. A practical approach to RT-qPCR-Publishing data that conform to the MIQE guidelines. *Methods* 50: S1–S5.

Wagenlehner F.M.E., G. Schmiemann, U. Hoyme, R. Fünfstück, E. Hummers-Pradier, M. Kaase, E. Kniehl, I. Selbach, U. Sester and others. 2011. National S3 guideline on uncomplicated urinary tract infection: recommendations for treatment and management of uncomplicated community-acquired bacterial urinary tract infections in adult patients. *Urologe. A.* 50: 153–169.

Yang S. 2003. Relative contributions of the AcrAB, MdfA and NorE efflux pumps to quinolone resistance in *Escherichia coli*. *J. Antimicrob. Chemother.* 51: 545–556.

Yasufuku T., K. Shigemura, T. Shirakawa, M. Matsumoto, Y. Nakano, K. Tanaka, S. Arakawa, S. Kinoshita, M. Kawabata and others. 2011. Correlation of overexpression of efflux pump genes with antibiotic resistance in *Escherichia coli* strains clinically isolated from urinary tract infection patients. *J. Clin. Microbiol.* 49: 189–194.

Yoshihara E. and H. Inoko. 2011. Method or agent for inhibiting the function of efflux pump *Pseudomonas aeruginosa*. http://www.google.com/patents/US7985410, 2016.02.10.