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Efflux pump induction by Quaternary Ammonium Compounds (QAC) and fluoroquinolone-resistance in bacteria

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ABSTRACT:

Biocides, primarily those containing quaternary ammonium compounds (QAC), are heavily used in hospital environments, and various industries (food, water, cosmetic, etc). To date, little attention has been paid to potential implications of QAC use in the emergence of antibiotic resistance, especially fluoroquinolone-resistant bacteria in patients and in the environment. QAC-induced overexpression of efflux pumps can lead to: i) cross-resistance with fluoroquinolones mediated by multidrug efflux pumps; ii) stress response facilitating mutation in the Quinolone Resistance Determining Region; iii) biofilm formation increasing the risk of transfer of mobile genetic elements carrying fluoroquinolone or QAC resistance determinants. By following the European Biocidal Product Regulation, manufacturers of QAC are required to ensure that their QAC-based biocidal products are safe and will not contribute to emerging bacterial resistance.

KEYWORDS

Quaternary Ammonium Compounds (QAC); fluoroquinolone; integron; efflux pump; antibiotic resistance; multidrug resistance.

Quaternary ammonium compounds (QAC) are among the most commonly used disinfectants in a number of fields of applications (Table 1) [1-3]. Over the past decade there has been a dramatic increase in the use of QAC: In Europe, in 2006, the market for biocides amounted to €10-11 billion, with an average growth of 4-5% per annum during the previous 15 years, and market expansion is predicted to continue (http://www.pan-europe.info/campaigns/biocides). QAC are now commonly found in consumer products, such as washing liquid, home surface disinfectant, toiletries, in Western Europe and North America. The main QAC currently used are benzalkonium chloride (BC), stearalkonium chloride, and didecyldimethylammonium chloride (DDAC) (Table 2) [3-5]. Their impact may not be limited to their area of use, as QAC ultimately reach the environment via waste water and may remain there for a long time, due to their poor biodegradability [6,7]. In Europe, the European Biocidal Product Regulation [8] states in several articles that the possible effect of a biocidal product on developing resistance and cross-resistance in bacteria needs to be evaluated. Evidence of bacterial developing antimicrobial resistance following the use of biocide was reviewed by the Scientific Committee on Emerging and Newly Identified Health Risks [9], and more recently by Maillard *et al.* [10]. There are a number of definitions of bacterial 'resistance', to biocides. The following definition has been proposed: "'*a change in susceptibility to a microbicide that renders it ineffective against a micro-organism that was previously susceptible to that microbicide*' [10], but this definition does not necessarily apply to the existing literature that is cited in this review. A number of papers are defining resistance to QAC as an increase in MIC, although this should now be viewed as a reduced susceptibility [10].

Fluoroquinolones (FQ) are potent broad-spectrum antibiotics that have been used in medical practice for the treatment of severe or resistant bacterial infections since the late 1980s. As their name suggests, they are derived from the quinolone family of antibiotics; quinolones themselves are synthetic constructs, developed by modification of 1-alkyl-1,8-naphthyridin-4-one-3-carboxylic acid [11]. Fluoroquinolones differ from quinolones by the replacement of the eighth carbon atom of the backbone with a nitrogen atom and the addition of a fluorine atom at the sixth position, giving them more potent antibiotic action and a broader spectrum of activity [12]. FQ are potent inhibitors of bacterial type II topoisomerases, which are essential enzymes involved in key cellular processes, including DNA replication [13-15]. Their spectrum of efficacy against a wide range of Gram-positive and Gram-negative pathogenic bacteria has led to their widespread use worldwide [16]. Yet, 30 years after their introduction, resistance levels have dramatically increased worldwide [17].

All bacteria have multidrug transporters or bacterial drug efflux pumps inserted into the cytoplasmic membrane that can remove toxic substances from the cytoplasm and from the cytoplasmic membrane [18]. The major clinically relevant efflux systems in Gram-negative bacteria belong to the resistance-nodulation-division (RND) superfamily (e.g. AcrAB-TolC in *Escherichia coli*, Mex-OPr in *Pseudomonas aeruginosa*), and are typically composed of a cytoplasmic membrane pump, a periplasmic protein and an outer membrane protein channel [19]. In the case of Gram-positive bacteria, the major facilitator superfamily (MFS; e.g., Bmr and Blt in *Bacillus subtilis* and NorA in *Staphylococcus aureus*), and the ATP-binding cassette (ABC) transporters are major players [19]. These efflux pumps can remove various

antibiotics and are then called multidrug resistance (MDR) pumps [18,20,21]. The regulation of efflux pump genes is complex and controlled by both dedicated regulators and multiple global regulators, which also control expression of other genes [22].

Commonalities exist regarding the concomitant use of FQ and QAC in industrial and hospital environments, and in both cases, resistance to FQ is increasing. In agribusiness, FQ are administered to treat infections in animals, and QAC are used as surface disinfectants in farms and in processing plants. In hospitals, QAC are used as surface disinfectant, and FQ is among the first two classes in terms of antibacterial agent consumption, second only to beta-lactams. QAC and FQ can persist in various environments. They have been detected in sewage sludge, river water and soil [23-25]. Class 1 integrons encoding efflux pumps were identical in clinical samples [26-28] and those isolated from soil and freshwater biofilms. The efflux pumps play an important role in increasing MICs, both for QAC that FQ, and the hazard/risk of biocide use leading to the selection of antibiotic-resistant bacteria and their dissemination is of increasing concern (http://ec.europa.eu/health/opinions/en/biocides-antibiotic-resistance/l-3/1-definition-antimicrobials.htm). In the meantime, WHO developed a list ranking antimicrobial classes according to their importance for public health. Fluoroquinolones were viewed as one of the highest priority for risk analysis [29].

The aim of this review was to present documented interactions between the use of QAC and the emergence of fluoroquinolone resistance in bacteria, focusing on three main pathways: i) “cross-resistance” of QAC and FQ mediated by multidrug efflux pumps, ii) QAC-induced stress responses that trigger mutation in the quinolone resistance-determining region (QRDR) and iii) synergistic effect on biofilm formation,

facilitating the transfer of mobile genetic elements carrying FQ or QAC resistance determinants.

- **Cross-resistance of QAC and FQ mediated by multidrug efflux pumps**

Overexpression of efflux pumps is hypothesised to mediate cross-resistance of FQ and QAC [30]. QAC can directly induce the expression of efflux pumps, or promote mutations within the regulators of efflux pump genes (Figure 1) [31-37]. Indeed, some strains carrying specific mutations at target sites are no longer resistant to FQ if efflux pumps are inactivated [38-40]. Efflux pumps may also play a role in the impact of mutations. Oethinger *et al.* showed that in the absence of the AcrAB efflux pump, topoisomerase mutations had no significant impact on FQ resistance in *E. coli* [38].

In Gram-negative bacteria, two of the best-studied RND-type MDR-pump systems are AcrAB-TolC or OqxAB in *E. coli*, SdeXY of *S. marcescens,* and Mex-Opr [20], and PmPM pumps in *P. aeruginosa,* MepA and NorA of *S. aureus* (Figure 2) [24,41-47]. In *E. coli*, AcrAB-TolC is present in wild-type bacteria, and its overexpression induces resistance to quinolones and QAC, as outlined below. Buffet-Bataillon *et al.* [48] observed an epidemiological association between high minimal inhibitory concentrations (MICs) of QAC and antibiotic resistance, without previous exposure to QAC. The efflux pump inhibitor phenyl-arginine-b-naphthylamide (PAbN) reduced the MICs of ciprofloxacin (CIP) and QAC, but remained ineffective in reducing the MIC of others antibiotics tested [48]. In this work, AcrAB-TolC system explained the association between resistance to FQ and QAC. Other works showed the induction of various efflux pumps in Gram-negative bacteria by antibiotics or by QAC [30,49-53]. For example, *Serratia marcescens* gained resistance to

cetylpyridinium chloride, BC and antibiotics by expressing the SdeAB efflux pump upon exposure to cetylpyridinium chloride [51]. In *P. aeruginosa*, primary intrinsic resistance to FQ is due to MexAB-OprM as well as to MexXY-OprM [53]. The RND-type MexCD-OprJ multidrug efflux pump is induced by norfloxacin and by ADBAC [49]. In *P. aeruginosa,* ADBAC, FQ, ethidium bromide, and tetraphenylphosphonium chloride were all substrates for PmpM, a multidrug efflux pump belonging to the Multidrug and Toxic Compound Extrusion (MATE) family [50]. An active role of efflux pump activity in conferring adaptive and cross resistances against CIP and BC has been shown in *P. aeruginosa* isolated from food products [30]. *Burkholderia cepacia* developed stable tolerance to benzethonium chloride with cross-resistance to FQ, associated with enhanced efflux, reduced swarming mobility and increased biofilm formation [52]. Recently, Webber *et al.* [54] exposed *Salmonella enterica* serovar Typhimurium to biocides and showed mutations in *ramR*, which encodes the local repressor of *ramA*, encoding a transcriptional activator that regulates the AcrAB-TolC MDR efflux system.

The same observations were made with Gram-positive bacteria, including *Listeria monocytogenes,* a major agent for severe food-borne illness, or *Staphylococcus aureus*, an important cause of nosocomial infections. Mutations in efflux systems of *L. monocytogenes* strains are responsible for the multidrug resistance phenotype of strains selected on CIP or ADBAC [55]. Other authors also observed that reduced susceptibility to biocides in staphylococci was associated with quaternary ammonium compound (*qac*) gene-encoding efflux proteins (*qacA, qacB, qacC, qacG*) [56,57].

However, if many studies are in accordance with the concept of cross-resistance to QACs and FQ, others are not so supportive. Furi *et al.* [57] showed that

9.5% of clinical isolates of *S. aureus* carried known genes associated with ethidium bromide efflux pump and reduced susceptibility to biocides. Fine characterization of the substrate specificity of these pumps associated (i) mutations of the promoter region of *norA* with a 2-fold increase in MIC to BC, (ii) the presence of the plasmid-encoded MFS pumps QacA and QacB with a 4-fold increase in MIC of BC, and (iii) the plasmid-encoded SMR efflux pumps QacC and QacG with a 2-fold increase in MIC to BC. Regarding cross-resistance to antibiotics *in vitro*, mutation of the *norA* promoter conferred cross-resistance to CIP and norfloxacin (NOR), but not all clinical isolates with *norA* promoter mutations were resistant to CIP, and none of the plasmid encoded efflux pumps conferred resistance to antibiotics. Such a study highlights the importance of future clinical work to validate these experiments.

- **Stress responses that trigger mutation in the quinolone resistance-determining region (QRDR)**

FQ target DNA gyrase and topoisomerase IV in a broad range of bacteria, by inhibiting their control of supercoiling within the cell, resulting in impaired DNA replication (at lower concentrations) and cell death (at lethal concentrations) [58,59]. An important mechanism of bacterial resistance to FQ is due to mutation(s) in one or more of the genes that encode the primary and secondary targets of these drugs, type II topoisomerases (*gyrA, gyrB, parC*, and *parE*). The region where mutations arise in these genes that encode FQ resistance is a short DNA sequence known as QRDR [60,61]. Mutations in QRDR, resulting in amino acid substitutions, alter the target protein structure and subsequently the FQ binding affinity of the enzyme, leading to drug resistance [16,62]. Recently, Weber *et al.* [54] described that

Salmonella enterica serovar Typhimurium to QAC and selected antibiotic-resistant mutants: genes in the QRDR region (*gyrA*) and in *rpoA,* encoding the RNA polymerase alpha subunit, were altered [54].

The SOS response is an inducible pathway governing DNA repair. Two key proteins govern the SOS response: LexA (a repressor) and RecA (an inducer). Whilst the SOS response was initially recognised as regulating DNA damage repair, its broader role is now well established. The SOS error-prone polymerases that enable translation and DNA synthesis also promote an elevated mutation rate that generates genetic diversity and adaptation, hence facilitating the emergence of antibiotic resistance [63,64]. QAC are strong cationic surface-active chemicals and could be hypothesised to trigger a SOS response following their physical interaction with the bacterial membrane. However, there are no studies that have directly investigated at effect of QAC and bacterial SOS response. Ceragioli *et al.* [65] reported that *Bacillus cereus*, upon exposure to selected concentrations of BC (0.5 to 7 mg/L), induced genes involved in the general and oxidative stress responses. Although this study did not look directly at SOS response, others have observed that exposure to oxidative stress can activate the SOS response [66]. A better evidence of the direct effect of QAC exposure on SOS response is needed.

If QAC are indeed significant triggers of the SOS response, this would increase mutation rates, which may promote the emergence of mutations in topoisomerases. However, these assumptions are not supported by robust scientific evidence to date, except on that of McCay *et al.* [67], who described a *P. aeruginosa* mutant highly adapted to ADBAC with an increased resistance to CIP (0.125 to 32 mg/L) in 33 generations elapsed in culture enrichment. This was due to mutations in

QRDR of *gyrA* and in genes *mexR* and *nfxB* that encode repressors of *mexAB-oprM* and *mexCD-oprJ*, respectively.

- **The impact of QAC on efflux pumps, SOS responses, and biofilm formation, may facilitate the transfer of mobile genetic elements carrying FQ or QAC resistance determinants between bacteria**

Recent research showed that QAC, efflux pumps expression and biofilm formation are connected processes [68-70] (Figure 1). QAC have been shown to induce expression of efflux pumps [31-37]. Baugh *et al*. demonstrated that both genetic inactivation and chemical inhibition of efflux pumps resulted in transcriptional repression of genes involved in biofilm matrix production, leading to biofilm formation inhibition [62]. Similarly, a significant increase of efflux pump activity was observed with changes in their biofilm formation potential [68]. Pagedar *et al.* [68] demonstrated that QAC can trigger biofilm formation, highlighting the role of adaptive response to FQ and BC. These findings provided an insight into the process of conversion from non-resistant to resistant isolates in parallel with biofilm formation following adaptation to antimicrobial agent exposure [68]. Ebrahimi *et al.* [71] documented the effects of BC on planktonic growth and biofilm formation in *E. coli, Salmonella* sp., *S. aureus,* and *Streptococcus agalactiae*, and found that in all the bacteria tested biofilm formation increased with decrease of BC concentration. These results were confirmed for *L. monocytogenes* exposed to disinfectants such as BC, which are commonly used to control contamination in food processing plants. However, Ortiz *et al.* [72] also showed that effect of sub-MIC of BC on biofilm production by *L. monocytogenes* might differ between strains with different MICs, and even between resistant strains with similar MICs but different genetic determinants of

BC resistance. One of the mechanisms involved in reducing biofilm susceptibility to antimicrobials is the presence of bacterial persisters [73]. The AcrAB-TolC MDR efflux pump has been shown to lower the intracellular concentration of FQ in *E. coli,* and to increase the level of surviving persisters and their tolerance to FQ [74]. Stress responses may also act as general activators of persister formation, via the toxin-antitoxin system TisB, inducing the entry of bacteria into dormancy [70,74]. Although QAC can trigger efflux and cause bacterial stress, there is a need for studies showing a direct link between QAC exposure and increased levels of persisters in bacterial biofilms.

- **Several studies have also demonstrated that biofilm formation and horizontal gene transfer are connected processes.**

Interrelations between QAC, efflux pump expression and biofilm formation, were described above. Madsen *et al.* [75] also reviewed the relationship between biofilm formation and horizontal gene transfer. Biofilms can enhance the host range of mobile genetic elements carrying FQ resistance determinants that are transferred horizontally [75], or *qac* gene cassettes [76]. Horizontal gene transfer can occur by conjugation, transformation or transfection, through mobile genetic elements (integrons), or plasmids. Both conjugative plasmid DNA transfer and transformation induce the SOS response [77-79]. Whereas both conjugation and transformation induce the SOS response, the latter also activates integrase genes involved in DNA transfer and recombination [80]. Integrons are genetic elements capable of integrating genes by a site-specific recombination system catalysed by an integrase and class 1 integron incidence was shown to be significantly higher for populations that were preexposed to QACs [81,82]. Guérin *et al.* [83] found that LexA controlled

expression of most integrases and consequently regulated cassette recombination [83]. Gaze *et al.* [84] propose that the integration of gene cassettes conferring resistance to FQ agents within integrons can be only a matter of time.

The integrating genes are exogenous genes, including *qnr, aac(60)-Ib-cr, qepA,* and *oqxAB*. The *qnr* genes have originated in the chromosomes of water-dwelling or other environmental organisms [85]. The environmental presence of *qnr* gene cassette on *qac*-containing integrons can contribute to the emergence of FQ resistance. Genes *qnrA, qnrB, qnrC, qnrD, qnrS,* and *qnrVC* code for proteins of the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition. Data from a recent structural analysis of a Qnr protein suggest that resistance to FQ is achieved by the binding of Qnr protein to topoisomerase, which physically prevents the intercalation of the antibiotic [86]. The *qnr* genes generally confer modest protection against FQ [87,88]. The gene *aac(60)-Ib-cr* confers decreased susceptibility to CIP and NOR by acetylating the amino nitrogen on the piperazinyl substituent present in these drugs [87,88].The *oqxAB* and *qepAB* genes encode efflux systems transporters that can export FQ molecules. Carriage of these genes again confers modest increases in FQ MICs [89]. The 3′CS of class 1 integrons contained *qacED1* and *sul1*, which mediate low-level resistance to QAC: *qacED1* encodes an efflux pump belonging to the small multidrug resistance family (SMR) and to sulfamethoxazole (*sul1*) [90-92]. Zou *et al.* [93] showed that the *qac* and *sugE*(p) genes were highly associated with FQ resistance among *E. coli* isolated from retail meats.

Unfortunately, only a few data are available about clinical examples of where quinolone resistance is clearly related to QAC use. In clinical *E. coli* isolates, Buffet-

Bataillon *et al.* [48,92] demonstrated an epidemiological relationship between higher MIC values of QACs and antibiotic resistance, involving AcrAB-TolC system and class 1 integrons. They observed the presence of *dfrA/sul1* and *qacEΔ1* gene cassettes correlated with resistance to co-trimoxazole and high MICs of QACs, with overexpression of *tolC, marOR* and *soxS*. Use of an inhibitor of efflux pumps reduced the MICs of ciprofloxacin and QACs, suggesting that extrusion of CIP and QACs from bacteria depends on the AcrAB-TolC system. Sidhu *et al.* [94] studied 61 strains of *S. aureus* and 177 coagulase-negative staphylococcal strains, isolated from the blood of patients with bloodstream infections and from the skin of both children under cancer treatment and human immunodeficiency virus-infected patients. The MIC analyses revealed that 118 isolates (50%) were resistant to QAC-based disinfectant BC. The frequencies of resistance to a range of antibiotics, including CIP, were significantly higher among BC-resistant staphylococci than among BC-sensitive staphylococci. Only *qacA/B* and *blaZ* probes where tested (they hybridized to the same plasmid in 19 (24%) staphylococcal strains), but the higher frequency of antibiotic resistance among BC-resistant strains indicates that the presence of either resistance determinant selects for the other during antimicrobial therapy and disinfection in hospitals.

- **Conclusions**

Resistance to FQ is mainly related to antibiotic use and misuse [95], but the evidence presented above could potentially be applicable to other antimicrobial agents, sharing the same resistance and survival mechanisms. We may have underestimated the effects of non-antibiotic antimicrobials, such as QAC, with an

extensive use in biocidal products [9,96]. The observations listed in this review support that various mechanisms of resistance that may contribute to cross-resistance between biocide and antibiotics, including overexpression of efflux pumps, biofilm formation, and spontaneous mutations [16,53]. Concerning the use of QAC some of the evidence between QAC exposure and resistance to FQ can be circumstantial and more clinical studies are needed to confirm or not QAC role in triggering antibiotic resistance. The overexpression of efflux pumps facilitates the horizontal transfer of mobile genetic elements carrying FQ resistance determinants (*qnr, aac(60)-Ib-cr, oqxAB, qepAB*) in Class 1 integrons (*QacED1*). However, there remain important gaps in our understanding of the mechanisms involved: Clinical studies are warranted to validate the *in vitro* experiments, and decipher the impact of QAC use on antimicrobial resistance, including to FQ, in patients and in the environment.

- **Future perspective**

In Europe, the Biocidal Product Regulation (No 528/2012) [8] is now requiring that manufacturers provide evidence that their biocidal products will not promote antimicrobial resistance. In the US, the Food and Drug Administration recently required manufacturers of antimicrobial hand wash to provide evidence that their products had no effect on the emergence of bacterial resistance to antimicrobials [84]. Although there are no standard to measure the impact of increasing use of biocidal products on the emergence of microbial resistance, Knapp *et al.* [97] developed such a test and validated its use with biocide and biocidal products. However, the proposed test does not investigate the effect of biocide usage and gene transfer.

Understanding the origins, evolution, and mechanisms of transfer of resistance elements is important for our ability to adequately address this public health issue.

EXECUTIVE SUMMARY

Quaternary Ammonium Compounds (QAC)

- Over the past decade there has been a dramatic increase in the use of QAC and their fields of application.
- The European Biocidal Product Regulation requires evidence of the possible effect of biocide on developing resistance and cross-resistance in bacteria.

Fluoroquinolones (FQ)

- Multidrug resistance and decreased susceptibility to FQ are now widespread
- FQ is viewed as one of the highest priority for risk analysis by WHO.

QAC and Efflux pumps and FQ-resistance in bacteria

QAC induce the overexpression of efflux pumps that could potentially lead to:

- i) a cross-resistance of QAC and FQ mediated by multidrug efflux pumps,
- ii) a stress response that triggers mutation in the quinolone resistance-determining region (QRDR), that is only supported by some solid scientific evidence to date.
- iii) increasing the number of persisters in biofilm, and facilitating the transfer of mobile genetic elements carrying FQ or QAC resistance determinants.

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Figure and Table legends

Figure 1: Summary of the impact of quaternary ammonium compounds (QAC) use on resistance to fluoroquinolones. *QRDR: quinolone resistance-determining region.

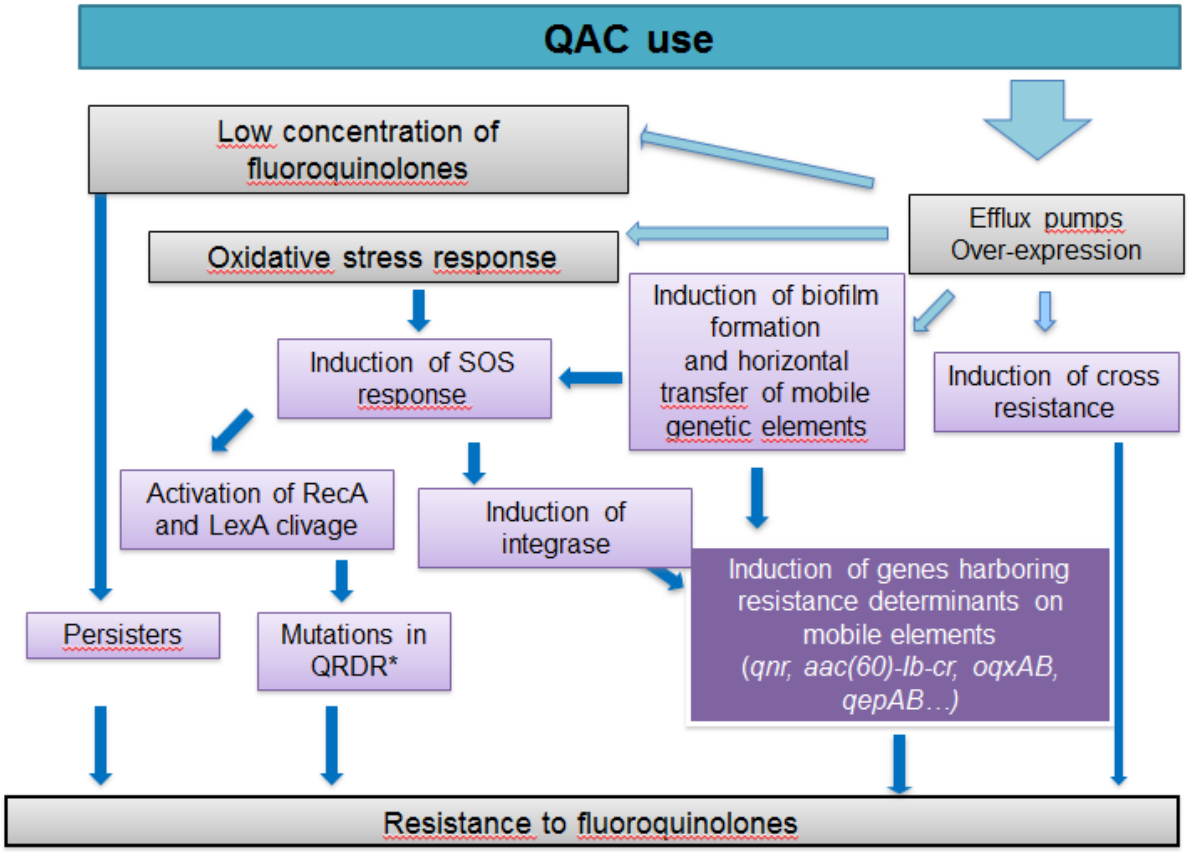


Figure 2. Schematic illustration of the main types of multidrug-resistance efflux pumps (MDR) involved in extrusion of QAC and quinolones in a) Gram positive and b) Gram-negative bacteria [24,42-47]. Hatched: the efflux pumps encoded by plasmid genes

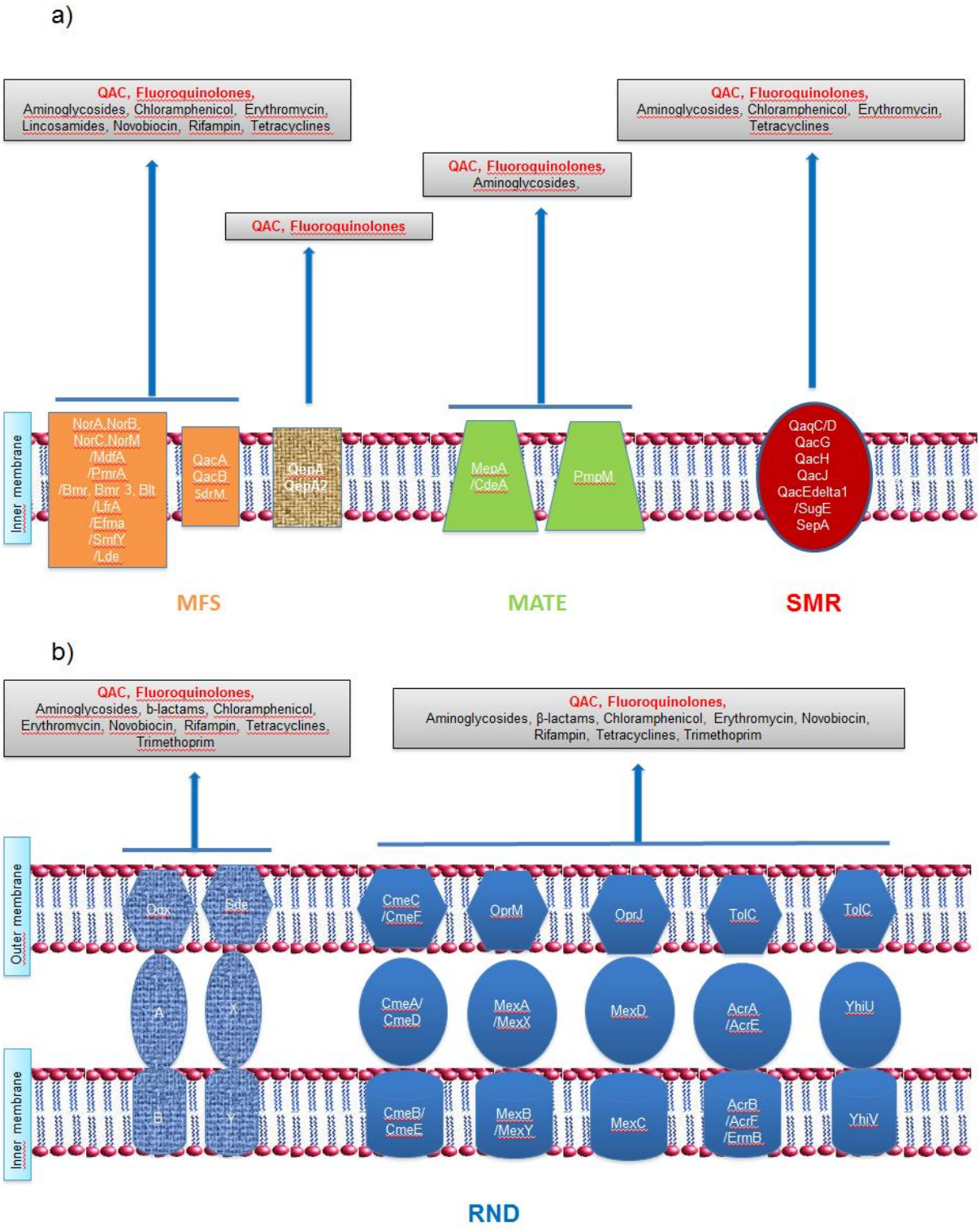
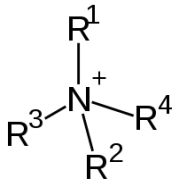
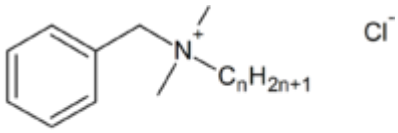
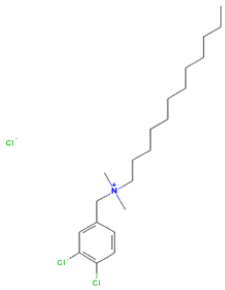

a)
QAC, Fluoroquinolones, Aminoglycosides, Chloramphenicol, Erythromycin, Lincosamides, Novobiocin, Rifampin, Tetracyclines
QAC, Fluoroquinolones, Aminoglycosides, Chloramphenicol, Erythromycin, Tetracyclines
QAC, Fluoroquinolones, Aminoglycosides,
QAC, Fluoroquinolones
Inner membrane
NorA,NorB, NorC,NorM /MdfA /PmrA /Bmr, Bmr 3, Blt /LfrA /Efma /SmfY /Lde
QacA QacB SdrM
QepA QepA2
MepA /CdeA
PmpM
QaqC/D QacG QacH QacJ QacEdelta1 /SugE SepA
MFS
MATE
SMR
b)
QAC, Fluoroquinolones, Aminoglycosides, b-lactams, Chloramphenicol, Erythromycin, Novobiocin, Rifampin, Tetracyclines, Trimethoprim
QAC, Fluoroquinolones, Aminoglycosides, β-lactams, Chloramphenicol, Erythromycin, Novobiocin, Rifampin, Tetracyclines, Trimethoprim
Outer membrane
Oqx
Sde
CmeC /CmeF
OprM
OprJ
TolC
TolC
A
X
CmeA/ CmeD
MexA /MexX
MexD
AcrA /AcrE
YhiU
Inner membrane
B
Y
CmeB/ CmeE
MexB /MexY
MexC
AcrB /AcrF /ErmB
YhiV
RND

Table 1: Fields of quaternary ammonium compounds (QAC) use [3,4].
Preservatives in pharmaceutical and cosmetic products : Eyewash/artificial tears, nose decongestant lotions, facial cleansers, acne treatment, sun protection creams and lotions, baby lotions, moisturisers, pain relief poultices or creams, hair conditioners, hair colour and styling products, make-up and make-up removal products, and hand sanitizers Supplement in commercially available alcohol-based hand rubs, which contain mainly ethanol, isopropanol or n-propanol, as well as in mouthwashes. *Benzalkonium chloride*
Cleaning and disinfecting farm buildings : poultry buildings (hatcheries), poultry cages, poultry house premises, poultry feeding equipment, poultry watering equipment, poultry equipment, poultry transportation vehicles, animal cages, animal living quarters, animal feeding and watering equipment, commercial egg treatment, hatchery equipment, incubators, hatchery premises, farm premises, agricultural equipment, shoe baths, and poultry processing equipment *Alkyl dimethyl benzyl ammonium chloride, Didecyl dimethyl ammonium chloride, Octyl decyl dimethyl ammonium chloride, Dioctyl dimethyl ammonium chloride*
Decontamination of healthcare devices and environmental surfaces *Benzalkonium chloride, Didecyl dimethyl ammonium chloride*
Waste water purification *Benzalkonium chloride*
Antifungal treatment in horticulture *Benzalkonium chloride*

Table 2: Different generations and chemical structures of quaternary ammonium compounds (QAC).

Generation quarternaries	Description
	The QAC are ammonium compounds in which four organic groups (R1-4) are linked to a nitrogen atom (N) that produces a positively charged ion (cation). In these QAC, the organic radical is the cation, and chlorine is usually the anion (X).
First generation quaternaries  n = 8, 10, 12, 14, 16, 18	The first generation of QAC are alkyldimethylbenzyl-ammonium chloride (ADBAC) ie Benzalkonium chloride with alkyl chains of 8 to 18 Carbons (C); Cetalkonium chloride= ADBAC with alkyl chains of 16 C; Stearalkonium chloride with alkyl chains of 18C.
Second generation quaternaries 	The second generation of QAC was obtained by substitution of aromatic rings in ADBAC by chlorine or alkyl distributions to get the products like to get the products like alkyldimethylethylbenzylammonium chloride; Chlorure d'aralkonium (Alkyl diméthyl-3, 4-dichlorobenzyl-ammonium chloride).
Third generation quaternaries	The third generation of QAC are a mixture of first and second generation, i.e., benzalkonium chloride and alkyldimethylbenzylammonium chloride.
Fourth generation quaternaries 	The Twin Chain QAC with chains that are dialkyl linear and without the benzene ring are the fourth generation of QAC ie dialkyl methyl amines as Dioctyl Dimethyl Ammonium Chloride, Didecyl Dimethyl Ammonium Chloride

	(DDAC).
Fifth generation quaternaries	The mixtures of the fourth generation (Dialkyl methyl amines) with the first generation (alkyl dimethyl benzyl ammonium chloride) represents the fifth generation