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Article

Third-Generation cephalosporins (3GC) and Fluoroquinolones (FQ) Co-Resistance in Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* (ESBL-Ec) from Clinical and Community Isolates

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Abstract. The growing AMR issue affects the current antimicrobial therapy recommendations, particularly for broad-spectrum antibiotics, like third-generation cephalosporins (3GC) and fluoroquinolones (FQ). Actually, the inappropriate use of both antibiotics in clinical and community settings increase the resistance of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-Ec). Although ESBL-Ec is used as a surveillance indicator of bacterial resistance, but currently studies related to 3GC-FQ co-resistance among clinical and community (including human and wastewater samples) based ESBL-Ec isolation, have not been widely carried out. The objective of this study was to analyze the possibility and mechanism of 3GC-FQ co-resistance among ESBL-Ec, in human clinical and communal isolates from previous published research. Out of 257 articles screened, four studies in accordance with our study are included in the analysis. The result indicated that ESBL-Ec derived from all sample sources had 3GC-FQ co-resistance. According to two studies reviewed, bla_{CTX-M} was the most predominant ESBL gene, while the FQ-associated resistant gene dominated by *qnr* family genes. Resistant genes and co-resistant ESBL-Ec can be spread rapidly through plasmids.

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1. Introduction

Globally, antimicrobial resistance (AMR) is regarded as a top priority concern, due to its fast growing effects on public health [1-2]. The growing AMR issue affects the current antimicrobial therapy recommendations, particularly for broad-spectrum antibiotics, like third-generation cephalosporins (3GC) and *fluoroquinolones* (FQ). The 3GC and FQ are suggested as 'first-line' treatments to prevent prescribing 'last-line' antibiotics such as carbapenem and colistin [3-4]. The misuse and overuse of broad-spectrum antibiotics, prolonged antibiotic use, and insufficient prevention of infection are some of the aspects that enhance the growth and dissemination of resistance [1-2], [5-6].

There have been 4.95 million antimicrobial resistance-related deaths worldwide, 1.27 million of which were directly related to infection [1]. By 2050, the antimicrobial resistance-related deaths predicted will increase more than 10 million [3], [7]. Not only in the hospital, which contains several dangerous infections, the association between community resistance and the use of antibiotics prescribed is also well characterized. Some resistant microorganisms play an important role as dangerous infection agents that cause antimicrobial resistance-related disease and death, such as *Shigella* spp*., Acinetobacter baumannii, Salmonella* spp*., Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* [8-12].

In terms of resistance to 3GC and FQ, the extended-spectrum beta-lactamase (ESBL)-producing bacteria must be a particular involvement. ESBL are enzymes that can break down 3GC, rendering them ineffective [11], [13]. ESBL is part of the cephalosporinases family, which is able to hydrolyze the beta-lactam ring in the penicillin group, 1-3 generation cephalosporins, and aztreonam. Beta-lactam ring hydrolysis prevents the antibiotic from binding to the receptor penicillin-binding proteins (PBPs) on the bacterial cell wall. This causes the antibiotics to become inactive [13]. Furthermore, the rate of FQ use is strongly correlated to the resistance to 3GC and other antibiotics. Then, it will improve the emergence of 3GC-FQ resistance.

In the study of co-resistance in Gram-negative isolates conducted by Sriyapai *et al*. (2022) [10], 3GC-FQ characterized in Salmonella and Shigella from clinical cases of intestinal infection. The study conducted in Thailand between 2011 and 2018. The result showed 97% and 98,1% Salmonella and Shigella were identified as ESBL producers, and cefotaxime (CTX)-resistant. Among the ESBL producer found the ciprofloxacin (CIP) and CTX or ceftriaxone (CRO) co-resistance isolates which have $bla_{\text{CTX-M-15}}$ and other ESBL and/or *AmpC* beta-lactamase genes, as well as FQ-associated resistance genes including an *aac(6')-Ib-cr,* also the mutation of both *gyrA* and *parC.* In addition, *qnrS* determinants were identified from the ESBL-producing Salmonella and Shigella isolates [10]. In another study using ESBL-producing *Klebsiella pneumoniae* isolated from adults in Malawi, $bla_{\text{CTX-M-15}}$ was the most commonly identified, but the FQ-associated resistance gene were less common [11].

The highest human gut colonizer, *E. coli,* particularly for the ESBL producer strain is used as an indicator of bacterial resistance surveillance, not only in the human sector, but also in the environmental sector [14]. ESBL-producing *Escherichia coli* (ESBL-Ec) are frequently resistant to different types of antibiotics, including aminoglycoside and FQ. Due to the adaptability of the *E. coli* genome, this species can rapidly develop a wide range of resistance mechanism, which can easily result in multidrug resistance [12-13]. It is important to determine and understand more about co-resistance, particularly 3GC-FQ co-resistance in ESBL-Ec.

Currently, studies related to 3GC-FQ co-resistance in ESBL-Ec from clinical and community (including human and wastewater samples) have not been widely carried out. The objective of this article was to review the possibility of 3GC-FQ co-resistance through ESBL-Ec, in human clinical

isolates and communities by synthesizing from phenotypical and molecular or genomic published studies, related to ESBL-Ec isolated from human clinical and community, samples, as well as wastewater samples. Knowing the co-resistance potential, the method of detection, and the mechanism of these antibiotic groups will be useful during the development of prevention and in reducing the emergence of resistance to 3GC-FQ.

2. Materials and Method

This review article contained scientific research findings from ScienceDirect (https://www.sciencedirect.com); PubMed (https://pubmed.ncbi.nml.nih.gov); and ProQuest (https://www.proquest.com). In order to find references for this article, systematic searches were done using search terms "((ESBL-producing E.coli OR ESBL-Ec) AND (Non ESBL-producing E.coli OR Non ESBL-Ec) AND (co-resistance OR co-occurrence OR co-selection OR co-existence) AND fluoroquinolones AND third-generation cephalosporins". From the search terms used, a total of 257 articles were found, then assessed for writing-related appropriateness.

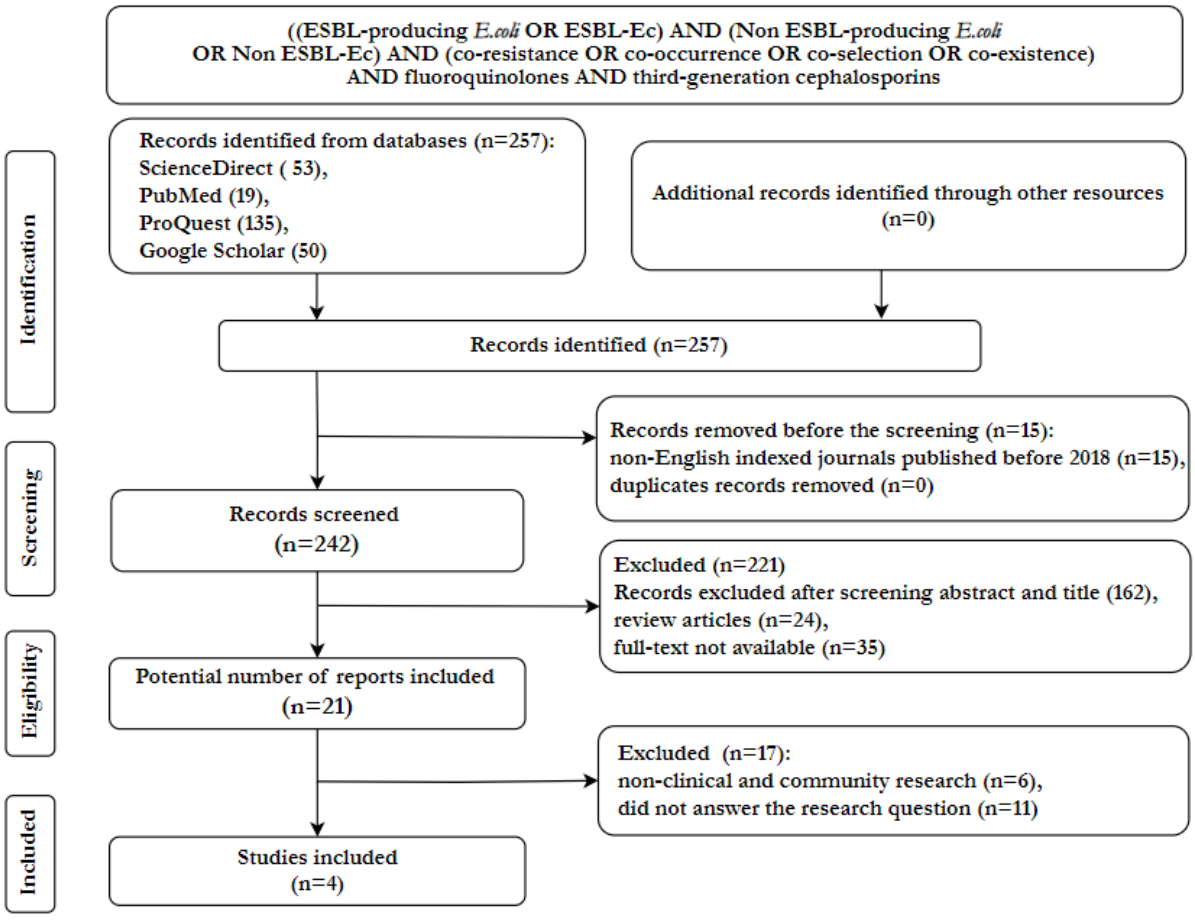

Figure 1. Summary flowchart of the articles included

The articles were subsequently screened using a database screener and Microsoft Excel based on the English-language indexed journal published between 2018-2023, duplication, relevance of the report's title, abstract, and the full text article. In the article screening process, we confirmed that the selected articles had to mention *E. coli*, ESBL-producing *E. coli* or ESBL-Ec in titles. Furthermore, the abstract and the entire articles selected had to discuss the phenotypic or genotypic investigation of ESBL-Ec resistance to 3GC-FQ. The target population of interest in the reviewed articles includes patients from human and wastewater settings. There were no restrictions on which countries conducted research. As a result, four reports were used in this article. In addition, there are several results in the form of related books and articles to complete relevant descriptions that meet the inclusive criteria and are in accordance with the purpose of writing. Figure 1 represents the study flow diagram of this review article.

3. Results and Discussion

Studies included in this article as a result of systematic screening are from countries in Asia, Europe and Africa, conducted by in vitro study design. Two studies used human clinical samples from Turkish and Ethiopian patients as representative research from Europe and Africa, while the other Asian population was conducted in Iran and Japan. Most studies used human sample types from clinical and community settings; only one study used a communal wastewater sample (Table 1).

Table 1. The 3GC-FQ resistance among ESBL-Ec from clinical and community isolates in several countries.

Country	Iran	Japan	Turkey	Ethiopia
Author and the year of publication	Halaji *et al.* (2020) [15]	Shibuki *et al. (*2023) [16]	Demirci *et al.* (2019) [17]	Sewunet *et al.* (2022) [18]
Specimen type	Human clinical isolates and community isolates	Community isolates and clinical wastewater isolates	Human clinical isolates	Human clinical isolates
Number of subject	46 isolates from KTP with the UTI group (nephrology private clinic) 65 isolates from non-KTP in the UTI group (3 laboratory centers)	279 municipal wastewater isolates, 37 hospital wastewater isolates	Urine samples from 28 inpatients and 23 outpatients at private university hospital	138 ESBL-Ec isolates from 1087 clinically suspected infection patients (including those with pneumonia, wound infection, diarrhea, and UTIs)
Phenotypic method to detect ESBL-Ec	DDST and automated Vitek System	Disk diffusion continued by PCR	DDST	DDST
Genotypic method to detect ESBL and FQ-Associated resistance gene	PCR	PCR	PCR	PCR

Results

Phenotype of 3GC and FQ resistance among ESBL-Ec compared to non ESBL-Ec (%)	significantly higher in clinical (3GC=100/11,5; FQ=54,33/34,6) and community (3GC=100/14; FQ=73,35/58,0) isolates (P<0.001)	100% 3GC resistance and >60% FQ resistance and in all 316 ESBL-Ec from communal and clinical wastewater	7,5%-100% 3GC-FQ co-resistance among ESBL-Ec from both inpatients and outpatients	ESBL+ ciprofloxacin (88,4%; 122/138)
ESBL genes	*bla*$_{TEM}$, *bla*$_{CTX-M}$	*bla*$_{TEM}$, *bla*$_{CTX-M}$	*bla*$_{CTX-M-15}$, *bla*$_{CTX-M-1}$	*bla*$_{CTX-M-27}$, *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-11}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{CMY-2}$, *bla*$_{CMY-42}$, *bla*$_{TEM-18}$, *bla*$_{SHV-12}$
FQ-associated resistance gene	NA*	*aac(6')-lb, qnrA, qnrB, qnrC, qnrD, qnrS*	NA*	*qepA, qnrB1, qnrB4, qnrB6, qnrS1*

*Notes: NA= Not Available
Description: KTP=Kidney Transplant Patients; PCR=Polymerase Chain Reaction; UTIs= Urinary Tract Infections; WGS=Whole Genome Sequencing.

The basic principle of the ESBL conventional phenotypic method is the ability of clavulanic acid to inhibit ESBL as well as the synergy induced by CTX and CAZ in combination. It is crucial to highlight that for both CDT and DDST methods, 3GC (CTX or CRO) and CAZ should be included in the test performed [13], [19-20]. Regarding the automatic system as a phenotypic ESBL method, there was the development system called VITEK 2 System which has 92% sensitivity to detect ESBL production. VITEK 2 System provides rapid detection of ESBL expression based on the concurrent evaluation of the inhibitory effects of antibiotics on bacterial growth [21].

In a study conducted by Halaji *et al.* (2020), 3GC and FQ resistance are higher in ESBL-Ec isolates compared to non ESBL-Ec isolates in both human clinical and community isolates. Antibiotic resistance of 3GC in ESBL-Ec clinical and community isolates dominated by ceftazidime (CAZ) and cefixime (CFM), while resistance of FQ has almost in the same level between CIP, nalidixic acid (NAL), ofloxacin (OFX), and norfloxacin (NOR) [15]. The ESBL produced by *E. coli* could lead to co-resistance when 3GC and FQ inappropriately administered as first-line antibiotics. CAZ, CFM and CIP were classified to "watch antibiotics" to treat a variety bacterial infections [6]. CAZ in combination with avibactam (AVI) utilized to treat serious Gram-negative bacterial infections, such as complicated urinary tract infections, and complicated intra-abdominal infections in hospitals [22-

23]. CIP is usually used for the gastrointestinal, urinary tract, and respiratory tract infections. CIP is one of the most widely prescribed antibiotics in primary health care facilities [5].

A study in Sendai, Miyagi prefecture, northeastern Japan by Shibuki *et al*. (2023) [16], ESBL genes and PMQR genes are present in ESBL-Ec isolates from both communal and hospital wastewater. This study shows 98,1% ESBL-Ec isolates carry 87% $bla_{\text{CTX-M}}$ and 98,1% $bla_{\text{CTX-M}}$ genes dominated by $bla_{\text{CTX-M-9}}$ (62,3%) from both wastewater sample types. There was no bla_{SHV} detected among all TGC (CTX, CXM, CAZ) resistant ESBL-Ec isolates. Total FQ (CIP)-associated resistance genes detected in this study dominated by *qnrS* (10,4%), *aac(6')-lb* (4,1%) and *qnrB* (1,9%) [16]. The results showed that $bla_{\text{CTX-M}}$ and bla_{TEM} were the two most common ESBL genes found in *E. coli* isolates. There is a possibility that the bla_{SHV} gene was not present in the ESBL-Ec isolates tested, or it may have been present at a lower prevalence compared to $bla_{\text{CTX-M}}$ and bla_{TEM}. Further research or specific studies may be needed to determine the reasons for the absence or lower detection of bla_{SHV} in these ESBL-Ec isolates [24-26].

The ESBL genes can be categorized into different families due to their diversity in nature. ESBL genes are found on plasmids, which are easily transmitted between and within different species of bacteria. The following ESBL genes can be found in the ESBL isolates including [27-31]:

1. TEM-type ESBLs: most TEM-type ESBLs are linked to cross-infections in hospitals. This gene encodes the ESBL derivatives, TEM-1, and TEM-2. Transposons that resemble Tn1-, Tn2-, or Tn3 typically carry this gene.
2. SHV-type ESBLs: SHV-type ESBLs are associated with cross-infections in hospitals. This gene is highly related to TEM-type. These gene variants differ only by a few amino acid substitutions.
3. CTX-M-type ESBLs: compared to TEM and SHV-type, CTX-M-type ESBLs have a significantly broader genetic diversity. The most dominant CTX-M type, such as $bla_{\text{CTX-M-55,}}$ has been discovered to be predominantly plasmid-mediated and to be present in many forms of conjugative plasmids with extremely diverse genetic settings. CTX-M-type ESBLs are responsible for the recent global increase in ESBL genes.
4. Other ESBLs: There are other ESBLs that are not classified into the above-mentioned families, such as PER, VEB, and GES.

Another study assessed in this review, conducted by Sewunet *et al.* (2022) [18] using clinical suspected infection patients at Jimma Medical Center, Ethiopia. This is the only study included in this review article, that used whole genome sequencing (WGS) method to determine resistant genes from samples collected. Unlike the other previous study, there are many variants of ESBL and FQ genes obtained which could not be found in the other study. The most commonly tested ESBL genes include $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} [24], but in this study, they also obtained CMY genes which encoding the AmpC cephalosporinases. The AmpC cephalosporinases is a naturally occurring enzyme that can hydrolyze cephalosporins and carbapenems [6]. However, when it is overproduced, it could offer resistance of broad-spectrum cephalosporins, including 3GC (CTX, CXM, CAZ and CRO). The overexpression of this enzyme can lead to the resistance of 3GC-FQ in the Northeastern France community [32].

There are two mechanisms that ESBL-Ec and other bacteria can use to develop resistance to FQ, which relate to the role of the FQ-associated resistance genes as follows [33-39]:

1. Plasmid-mediated quinolone resistance (PMQR)
   PMQR genes encode efflux pumps or enzymes that modify quinolones. Plasmids are responsible for carrying the PMQR gene. Plasmids are the small circular pieces of DNA that can be easily transferred between bacteria. *qnrA, qnrB, qnrS, qnrC, qnrD, oqx*AB, *qepA*, and *aac-(6')-Ib-cr* are examples of PMQR genes. PMQR genes can contribute to higher levels of FQ resistance in bacteria.
2. Quinolone resistance-determining region (QRDR) mutations

QRDR mutations occur in the topoisomerase IV and DNA gyrazse genes, which are FQ target genes. These mutations can reduce the binding of quinolones to their targets, thereby reducing the effectiveness of the antibiotics. Examples of QRDR genes include *gyrA, gyrB, parC*, and *parE*.

3. Interaction between PMQR genes and QRDR mutations
   Interaction between PMQR genes and QRDR mutations triggers greater levels of FQ resistance in bacteria. In clinical settings, PMQR genes might encourage QRDR mutations and contribute to the growth of high-level FQ resistance.

Regarding the FQ-associated resistance genes, in addition to obtaining *qnr* variants as PMQR, one research study conducted by Sewunet *et al.* (2022) [18] also obtained the *qepA* gene. This *qepA* gene is a plasmid-mediated efflux pump. It can be found in some strains of *E. coli* that significantly elevate resistance against quinolones. Along with the other resistance genes, including *aac(6')-Ib-cr*, *oqxAB*, and *qnr*, the *qepA* gene is frequently found in combination. This condition further complicates the medical management of infections caused by these bacteria. The expression of the *qepA1* gene is induced under antibiotic exposure, which can lead to increased resistance to CIP [34], [38], [40]. Other related efflux pump genes exist in *E. coli* are AcrAB-TolC efflux pump and *oqxAB* complex [35], [37], [41].

PMQR genes can modify quinolones or pump them out of the cell, while QRDR mutations can reduce the binding of quinolones to their targets. The interaction between PMQR genes and QRDR mutations can contribute to higher levels of quinolone resistance in bacteria [33], [36], [39]. There are six different PMQR gene types, including *aac (6')-Ib* and *qnr* family genes. The *aac(6')-Ib* generates the enzyme that modifies quinolones, while the *qnr* family genes altered the sites targeted by quinolones [16].

Both phenotypic and genotypic methods significantly contribute to addressing the possibility, prevalence, and antibiotic-resistant mechanisms in bacteria. With the exception of study conducted by Sewunet *et al.* (2022) [18], the other three articles presented in Table 1 used a PCR approach to detect both ESBL and FQ resistance genes [15-17]. PCR is a commonly used molecular technique to detect specific genes [42]. PCR amplifies the target DNA sequence, allowing for the identification of ESBL genes and FQ resistance genes. Multiplex PCR is a variation of PCR that allows for the simultaneous amplification of multiple target genes. It can be used to detect multiple ESBL genes, such as bla_{SHV}, $bla_{\text{CTX-M}}$, and bla_{TEM}, in a single reaction [24].

Based on the study of Sewunet *et al.* (2022) [18], the ESBL and FQ genes that are involved in the resistance of the isolates tested vary considerably. This shows the advantages of WGS compared to other methods. However, the WGS approach is the gold standard for typing bacteria and determining the interrelationships between strains. WGS has high accuracy in characterizing the complete genome structure of isolates tested. Nucleotide bases in the entire bacterial genome can be sequenced so that detailed information can be retrieved when it is analyzed using bio informatics [43]. Consequently, the ESBL and FQ genes that may be involved in 3GC-FQ co-resistance among isolates can be determined more completely than the other three studies presented in Table 1.

3GC-FQ co-resistance in ESBL-Ec isolates in this review is represented based on the result of the conventional or automatic phenotypical AST method. From the phenotypic method, 3GC and FQ resistance can be found. Then, from all four investigations reviewed, we observed that multiple ESBL-FQ genes are associated with 3GC and FQ resistance were transmitted into the same bacteria, based on the result of molecular or genomic analysis [15-18]. It is in line with reports of Baindara [44], regarding the co-resistance, which stated that when resistance genes are present on the same genetic component in the bacteria, such as plasmid, then the co-resistance will develop. In the water environment, co-resistance also can occur because of the co-location of the metals (Figure 2).

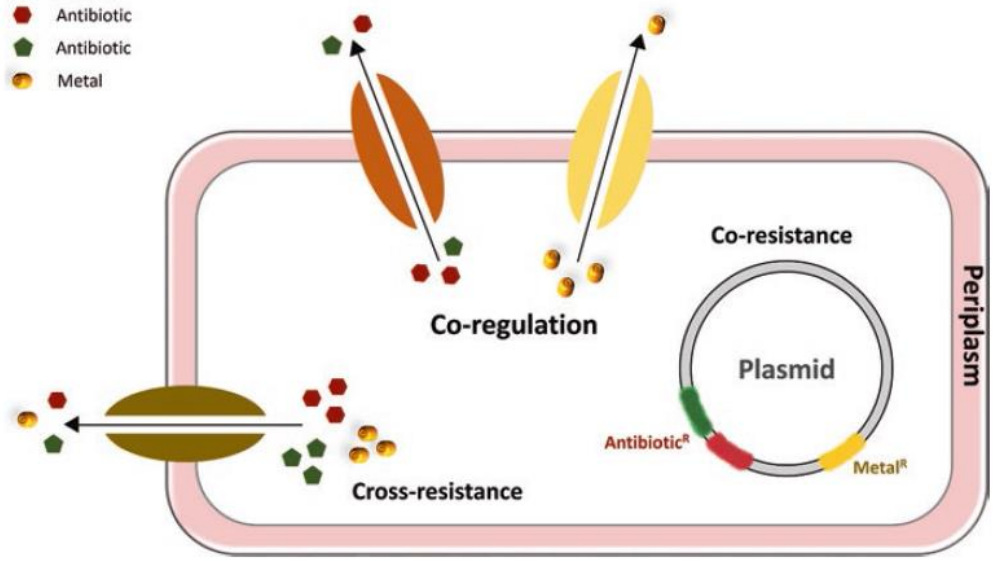

Figure 2. Bacterial co-resistance illustrated mechanism [44]

Even though based on Kotb *et al*. (2019) [33] regarding the co-resistance can also occur because of resistance gene mutation, but no one of four studies reviewed in this article has specifically discussed gene mutations related to co-resistance. Baindara (2019) [44] and many authors on the 'bacterial adaptation to co-resistance' topic reported that the presence of mutations at many genetic loci that influence various antimicrobials, as well as the transfer of a variety of resistant genes into the single strain bacteria, are characteristics of co-resistance. Co-resistance and co-selection significantly enhance the likelihood of bacterial survival and resistant genes. The co-resistant isolates that contain co-resistant genes are more dormant in nature; they have a regulation mechanism due to various factors, including the presence of a resistance mechanism [14], [44-45]. There are several explanations for why co-resistance bacteria might be dormant in nature:

1. Selection of pre-existing resistance
   Bacterial communities have an impact on the selection of pre-existing resistance. Bacterial interactions between species that occur in a community can influence the development and maintenance of resistance.
2. Evolutionary dynamics
   This refers to the evolutionary dynamics and effects of exposure to antibiotics, particularly in a clinical setting.
3. Breakdown of antibiotics
   For instance, when beta-lactamases which are enzymes that confer resistance, break down antibiotics, they increase the ability of resistant cells to detoxify their environment, creating conditions that facilitate the survival and dormancy of co-resistance bacteria.
4. Intrinsic resistance
   Some organisms are considered "intrinsically" resistant to one or more antimicrobials. This means that they have inherent mechanisms that make them naturally resistant to certain antibiotics.
5. Selective pressure
   On bacterial populations, human activities can exert selective pressure then leading to the enrichment of resistance determinants. This can result in the dissemination and persistence of co-resistance bacteria in the environment.

ESBL-Ec that carry co-resistance 3GC-FQ genes can be spread through the community, communal waters, and wastewaters, which then affect healthy people outside of the hospital setting as well. Consequently, healthy populations can carry clinically important antimicrobial resistant bacteria and antimicrobial resistance genes. Although carrier populations in the community are asymptomatic, they can act as a reservoir that is likely the source of much of the clinical resistance that occurs [14], [45].

4. Conclusion

ESBL from *E. coli* increases the co-resistance, particularly if it is encouraged by the use of 3GC and FQ as first-line antibiotics. ESBL and FQ-associated resistance genes, as well as ESBL-Ec resistant, can be spread through communal waters and affect healthy populations outside of the hospital setting as well. Even if there are no symptoms, healthy populations can carry clinically important antimicrobial resistant bacteria and antimicrobial resistance genes. There are only a limited number of published studies related to the co-resistance of 3GC-FQ among ESBL-Ec in the human sector, particularly related genomic studies. The majority of 3GC-FQ study is focused on the animal sector, but its correlation to the human and environmental sector also needs to be improved.

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