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Assessment of Bacterial Resistance Trend and Contributing Factors to Fluoroquinolone among Patients' Specimens Analyzed At International Clinical Laboratories in Addis Ababa

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This is to certify that the thesis prepared by Banchirega Mekuria, entitled "Assessment of bacterial resistance trend and contributing factors to fluoroquinolone among Patients' specimens analyzed at International Clinical Laboratories in Addis Ababa" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacoepidemiology and Social Pharmacy complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Introduction: *Fluoroquinolones are a group of antimicrobials which are widely used globally and thus microorganisms becoming resistant to them. As there is limited current data on specific microorganisms' resistance to fluoroquinolones in Ethiopia; this study aimed to generate information on the bacterial resistance against fluoroquinolones.*

Objective: *To assess the bacterial resistance trend to fluoroquinolone among patients' specimens analyzed at International Clinical Laboratories and contributing factors in Addis Ababa, Ethiopia*

Method: *A retrospective, cross-sectional study design was employed using records from microbiology laboratory registers of International Clinical Laboratories documented from January 2013 to December 2016. Descriptive analysis and inferential statistics were computed. In addition, qualitative study using phenomenological method was conducted in health facilities of Addis Ababa to explore the contributing factors for resistance from prescribers' and dispensers' perspective. A thematic analysis was used to analyze the qualitative data.*

Result: *The overall bacterial resistance to fluoroquinolones was 42.5%. The resistance was high against nalidixic acid (63.3%) followed by norfloxacin (44.4%). Enterococci and E.coli isolates were developing high level of resistance to ciprofloxacin and norfloxacin while enterococci was highly resistant to nalidixic acid (77.8%) followed by Pseudomonas (75.0%) isolates. In general, resistance to fluoroquinolone increases as patients' age increases. Resistance against fluoroquinolones in patients of age 60 and above years were more than 5 times AOR= 5.63 (4.71, 6.73) that of the resistance in patient's age less than 15 years. The resistance to fluoroquinolones increased from 40.4% in 2013 to 45.1% in 2014 and reached to the 49.0% in year 2015 and then declined to 46.3% in 2016(P <0.05). Key informants revealed that inappropriate practices of physicians, pharmacists and patients and incomplete diagnostic services contributed to fluoroquinolone resistances. Strengthen the regulation regarding over the counter sell of antibiotics, educating the public to improve awareness, promoting ethical practice would also help rationalizing the prescribing and dispensing practices, and instituting evidence based use of antibiotics are recommended as a solution.*

Conclusion: *The retrospective study showed high bacterial resistance to fluoroquinolones. There was an increasing trend of bacterial resistance with time. Key informants stated that inappropriate prescribing and over the counter sell of antibiotics as well as irrational use by patients contributed to bacterial resistance to fluoroquinolones.*

Recommendation: *The study suggests updating of health professionals on susceptibility pattern of bacteria and rational prescription to improve use of fluoroquinolones, Promoting ethical practice, improving the regulation and supervision on over the counter sell of antibiotics at private medicine retail outlet and continuous awareness raising and behavioral change communication has to be devised towards rational use to the community were some of the proposed containment mechanism for bacterial resistance to fluoroquinolones.*

Key word: *Fluoroquinolone, resistance, bacterial isolates, resistance trends*

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List of abbreviation

CDC	Center for Disease Control and prevention
C.jejuni	*Campylobacter jejuni*
DNA	Deoxy Ribonucleic Acid
E.coli	*Escherichia coli*
FQ	Fluoroquinolones
ICL	International Clinical Laboratories
MDRP	Multi Drug Resistant *Pseudomonas aeruginosa*
MRSA	Methicillin Resistant *Staphylococcus aureus*
N. gonorrhoeae	*Neisseria gonorrhoeae*
P. aeruginosa	*Pseudomonas aeruginosa*
PMQR	Plasmid Mediated Quinolone Resistance
QRDRs	Quinolone Resistance Determining Region
RNA	Ribo Nucleic Acid
S. aureus	*Staphylococcus aureus*
SPSS	Statistical Package for Social Scientists
UTI	Urinary Tract Infection
WHO	World Health Organization

1. Introduction

The modern era of antibiotics is started with the discovery of penicillin by Sir Alexander Fleming in 1928 (Yamamoto *et al.*, 2007). Since then, antibiotics have transformed modern medicine and saved millions of lives (Appelbaum, 2006). After 1940, new classes of antimicrobial agents were developed one after another, leading to a golden age of antimicrobial chemotherapy. The drugs have been developed to achieve better pharmacodynamics and as antimicrobial chemotherapy has been established and matured; more importance has been attached to the drug safety. Quinolone antimicrobials represent an example of drugs with improved pharmacodynamics and safety (Saga *et al.*, 2009).

Fluoroquinolone antimicrobial drugs are highly bioavailable, broad spectrum agents with activity against gram-negative pathogens, especially those resistant to other classes of antimicrobial drugs (Saga *et al.*, 2009). Nalidixic acid, the first drug of this class was introduced into clinical practice in the 1960s and was active only against gram negative bacteria, and its use was limited to urinary tract infections because it achieves only low blood concentrations and poor tissue distribution, and was metabolized rapidly in the human body (Saga *et al.*, 2009).

After that several fluoroquinolone antimicrobial drugs, including ciprofloxacin and norfloxacin in the 1980s, and then ofloxacin and levofloxacin, and more recently gatifloxacin and moxifloxacin were developed. Estimates from the late 1990s suggested that quinolones were the most prescribed antibacterial agent worldwide (Powers, 2004). Shortly after penicillin discovery, resistance became a substantial clinical problem, so that, by the 1950s, many of the advances of the prior decade were threatened (Fishman, 2006). The first case of methicillin-resistant *Staphylococcus aureus (MRSA)* was identified during that same decade, in the United Kingdom in 1962 and in the United States in 1968. Unfortunately, resistance has eventually been seen to nearly all antibiotics that have been developed (Appelbaum, 2006).

Even if a large number of companies in various countries have competed in the development of newer antimicrobial agents, the numbers of brand new drugs have been remarkably decreasing in recent years (WHO, 2010). In contrast, infectious diseases continue to attack human beings as

emerging and reemerging infectious diseases, opportunistic infectious diseases, and infection with drug resistant microorganisms. Emergence of antimicrobial resistance is a consequence of the use, overuse and misuse of antibiotics both in humans and animals. Antimicrobial resistance is one of the challenges in public health. Globalization has enabled the rapid spread of infectious agents, including those that are drug resistant. Diseases and disease agents that were once controlled by antibiotics are developing resistance (WHO, 2010).

Ineffective therapy due to antimicrobial resistance is associated with increased human suffering, lost productivity; increased costs associated with prolonged illness, more frequent hospital admissions and longer periods of hospitalization and death (WHO, 2010).Therefore, antimicrobial drug resistance needs an urgent global action since the condition has a great impact on the costs of health care provision. Antimicrobial resistance occurs everywhere but is particularly a concern in countries where prescription of antimicrobials is unregulated and where you can buy antibiotics over the counter. This is the case in many countries, including China and India, as well as many countries in Africa and Central and South America (WHO, 2010). Effective utilization of the current limited options is much more important under the dearth of new drugs on the market in country like Ethiopia. Therefore there is a need to understand the current pattern of fluoroquinolone resistance.

Fluoroquinolones are one of the antibiotics widely used in current clinical practice. Therefore this study will provide the trends and patterns of fluoroquinolones resistance and contributing factors for the resistance from the perspective of the prescribers and dispensers. The scope of the study was limited to the three commonly prescribed fluoroquinolones that is ciprofloxacin, nalidixic acid and norfloxacin. This will further help to guide clinical practitioners, policy makers and all other stakeholders for evidence based decision making.

2. Statement of the problem

Antibiotic resistant infections are already widespread across the globe. Among gram-positive pathogens, a global pandemic of resistant *S. aureus* and *Enterococcus* species currently poses one of the biggest threats (CDC, 2013).The global spread of drug resistance among common respiratory pathogens, including *Streptococcus pneumonia* and *Mycobacterium tuberculosis*, become an epidemic (Rossolini *et al.*,2014). Resistance is increasing, particularly to first-line, inexpensive, broad-spectrum antibiotics (WHO, 2001). From the late 1960s through the early 1980s, pharmaceutical industries introduced many new antibiotics to solve the resistance problem, but after that the antibiotic pipeline began to dry up and fewer new drugs were introduced (Spellberg *et al.*, 2014). Introduction of newer drugs such as fluoroquinolones has been followed relatively quickly by the emergence and dissemination of resistant strains (Okeke *et al.*, 1999). As a result, many decades after the first patients were treated with antibiotic, bacterial infections have again become a global threat (Ventola, 2015).

Infections with drug resistant organisms remain an important problem in clinical practice. If an improper antimicrobial agent happens to be chosen for the treatment of infection with drug-resistant microorganisms, the therapy may not achieve beneficial effect and may lead to worse prognosis. Beside this can result in costly prolonged illness and hospitalization, and use of other than first line drugs may also increase costs 100 fold making it unaffordable for many governments and patients especially in developing countries. In addition, in a situation where multidrug-resistant organisms have spread widely, there may be quite a limited choice of agents which may be more toxic for therapy. Considering this situation together with the increasing awareness of drug safety, we are now facing a situation of severely limited options among antimicrobial agents (John, 2001 and DACA, 2002).

Economic impact of antimicrobial drug resistance can be classified as direct and indirect cost. Direct Costs includes increased costs of disease surveillance, longer hospital stays, additional investigations, such as laboratory tests and x-rays, MDR infections lead to expensive and more toxic alternative treatments, greater likelihood of death due to inadequate or delayed treatment, increased annual investment and spending in healthcare, high for low income countries where as indirect Costs includes reduced quality of life and productivity, longer absenteeism for patients and care takers, increased costs to firms and leading to increased product prices, increased

burden and psychosocial impact, increases in cost of private insurance coverage, additional number of people living in poverty to 24 million in high AMR impact scenario ,AMR effects go beyond the health sector i.e. affecting GDP, 3.8% & may double by 2050 and educed output and trade in livestock and livestock products(world bank, 2016).

In developing countries misuse of antibiotics by health professionals, unskilled practitioners, and laypersons; poor drug quality; unhygienic conditions and inadequate surveillance accounting for spread of resistant bacteria (Okeke *et al.*, 1999).Therefore proper use of currently available antimicrobial drugs, as well as efforts to minimize the transmission and spread of resistant bacteria through appropriate infection control would be the first step in resolving the issue of resistant organisms. Different studies revealed that there is wide use of fluoroquinolones both in the hospital and in the community in developed as well as developing countries (Hooper,2000, Ohieku *et al.*,2013).The percentage of fluoroquinolone non-susceptible strains also showed an increasing trend globally (Suk *et al.*,2014). The occurrence of such resistance to commonly prescribed fluoroquinolone in developing countries like Ethiopia is not uncommon. For example, a study in Gonder University hospital showed that out of the total isolates, 93(61%) were found to be resistant to at least one antibiotic used for susceptibility testing. Forty eight (43.2%) of the isolates were multi-drug resistant. The resistance rate noted for both ciprofloxacin 17(11.1%) and ceftriaxone 15(9.8%) alarming (Anagaw *et al.*, 2012, Abejew *et al.* 2014 indicated that susceptibility to Nalidixic acid and ciprofloxacin *E.coli* isolate were 91(86.7) and 66(71.7%) respectively.

Information from routine susceptibility testing of bacterial isolates and surveillance of antibiotic resistance, which provides information on resistance trends, including emerging antibiotic resistance, is essential for clinical practice and for rational policies against antibiotic resistance. Therefore an updated knowledge of the prevailing non susceptibility patterns of microbial toward fluoroquinolones is important for the proper selection and use of these antimicrobial drugs as well as for the development of an appropriate policy to mitigate the problem. Since little is known about the current susceptibility pattern to fluoroquinolones, this study aims to investigate the susceptibility trend of bacterial isolates to fluoroquinolones as well as identify contributing factors to the resistance from the perspective of prescribers and dispensers in Addis Ababa Ethiopia.

3. Literature review

3.1. Background

The fluoroquinolone class of antimicrobials has had broad acceptance in hospitalized and community patients, and usage appears to be increasing (Hooper, 2000). With the fluorinated ones having a broaden activities and effectiveness in the treatment of a wide variety of infectious diseases. Although some members of the class (temafloxacin, grepafloxacin, and trovafloxacin) have been withdrawn or restricted because of adverse events, new members continue to be developed and approved (gatifloxacin and moxifloxacin) (Low *et al.*, 1998).

Agents like ciprofloxacin, ofloxacin, norfloxacin, pefloxacin and lomefloxacin regarded as second generation quinolones have an activity against expanded gram-negative, some gram-positive as well as atypical bacterial pathogen (Dana *et al.*, 2000). Agents like levofloxacin, gatifloxacin, sparfloxacin and moxifloxacin have increased gram-positive activities. The fourth generation like trovafloxacin, clinafloxacin and gemifloxacin has additional activity against anaerobic bacteria (Ambrose *et al.*, 1997; Dana *et al.*, 2000). This fact, plus the convenience of dosing suggests that use will increase (Hooper, 2000).

Many factors including their wide spectrum continue to justify their increased utilization in any given region. Their high oral absorption profile and high bioavailability as well as a serum concentration that compares well with those of intravenous administration continue to favor their frequent orderings. The fluoroquinolones also have large volume of distribution and concentrate in tissues at levels that exceed serum drug concentration and leading to high concentration in tissues of renal, lung, prostate, bronchial, nasal, gall bladder and genital tract making them useful in the treatment of infections in these areas (Ohieku *et al.*, 2013).

Uncomplicated urinary tract infections are most times treated empirically, particularly in areas where clinicians can recognize resistance patterns of uropathogens in the community. But this can pose challenges in most other places, thereby making it difficult for most appropriate antimicrobial agent to be chosen. Antibiotic selection for urinary tract infection can depend on many factors such as the allergy history of patients, treatment cost, tolerability of the treatment,

previous antibiotic therapy, and the prevalence of resistance in the community. All these factors appeared to favor fluoroquinolones use in many regions (Ohieku *et al.*, 2013).

However, the fluoroquinolones have been overused in many quarters leading to the development of resistant of both gram positive and gram negative bacteria. Experts recommended that physician should obtained information on local resistance rates, and ongoing local, regional and national surveillance be conducted to monitor changes is susceptibility to uropathogens and suitability of empiric therapy recommended (Ohieku *et al.*,2013).

3.2. Mechanisms of Fluoroquinolones Action

Fluoroquinolone inhibit deoxy ribonucleic acid (DNA) synthesis. Inhibition appears to occur by interaction of the drug with complexes composed of DNA and either of the two target enzymes, DNA gyrase and topoisomerase IV. These enzymes are structurally related to each other, both being tetrameric with pairs of two different subunits. The GyrA and GyrB subunits of DNA gyrase are respectively homologous with the ParC and ParE subunits of topoisomerase IV. Both enzymes are type 2 topoisomerases, which act by breaking both strands of a segment of DNA, passing another segment through the break, and then resealing the break. For DNA gyrase, this topo isomerization reaction results in introduction (or removal) of DNA super coils, thus affecting the negative super coiling of DNA necessary to initiate DNA replication and remove positive supercoils that accumulate before an advancing replication fork. For topoisomerase IV, the topo isomerization reaction results in separation of the interlocking of daughter DNA strands that develop during replication; this facilitates the segregation of daughter DNA molecules into daughter cells. In both cases, fluoroquinolones appear to trap the enzyme on DNA during the topo isomerization reaction, forming a physical barrier to the movement of the replication fork, ribonucleic Acid(RNA)polymerase, and DNA helicase. The collision of the replication fork with these trapped complexes triggers other poorly defined events within the cell that ultimately result in cell death (Hooper, 2001).

3.3. Uses of fluoroquinolones

The introduction of the fluoroquinolones provided clinicians with a class of broad spectrum agents applicable to a range of gram negative infections including urinary tract infections, gastrointestinal infections, respiratory tract infections including tuberculosis, sexually transmitted diseases, bone and joint infections, and infections of the skin and soft tissue. They have also been used for prophylaxis in neutropenic patients with cancers, in cirrhotic patients at risk for spontaneous bacterial peritonitis, and in urologic surgery (Alekshun *et al.*, 1999). Targeted microorganisms include the family *Enterobacteriaceae, Haemophilus spp., Neisseria spp., and Moraxella spp.*, which are highly susceptible to these agents, as well as important nosocomial pathogens such as *Pseudomonas aeruginosa* and *Acinetobacter spp*. fluoroquinolones are less active but still clinically useful against *Legionella* species (Poole ,2000).

Urinary tract infections

E. coli, other *Enterobacteriaceae* and *Enterococcus spp*. are the primary etiology of uncomplicated UTIs, with *E. coli* accounting more than 75% of isolates (Schito *et al.*, 2009). Quinolones have been widely used for the treatment of UTI because of their in vitro activity and high efficacy, especially in acute pyelonephritis and in catheter associated UTIs (Gupta *et al.*, 2011). However, the increased use of quinolones has been associated with increased rates of quinolone-resistance in clinical uropathogens (Suk *et al.*, 2014)

Respiratory tract infections

S. pneumoniae is a major cause of community acquired pneumonia, and guidelines for empiric antibiotic choices always list anti-*pneumococcal* antibiotics, including respiratory quinolones (Song *et al*., 2009). Therefore, respiratory quinolones such as levofloxacin, moxifloxacin, and gemifloxacin are selectively recommended for the treatment of patients having community-acquired pneumonia. As the use of quinolones increased, fluoroquinolone resistant *S. pneumoniae* has emerged in many countries and increased in some hot spots such as Canada, Spain, and Hong Kong (Low, 2004).

Intra-abdominal infections

Intra-abdominal infections are usually caused by mixed aerobic and anaerobic microorganisms, and the major pathogens in community-acquired intra-abdominal infections are coliforms *(Enterobacteriaceae,* especially *E. coli*) and *Bacteroides fragilis*. Among quinolones, moxifloxacin as a single agent therapy or a combination of metronidazole with ciprofloxacin or levofloxacin has been recommended for the treatment of mild to moderate community-acquired intra-abdominal infections. Combination therapy with metronidazole and quinolones is an option for the patients with high-severity community-acquired intra-abdominal infections (Solomkin *et al.*, 2010).

Skin and skin structure infections

The most common pathogens in skin and skin structure infections are *S. aureus* and *Streptococcus pyogenes.* They are also major pathogens in complicated skin and skin structure infections with poly microbial etiology that also include gram-negative organisms and anaerobes. The quinolones have antibacterial activity for many of these pathogens, excellent oral bioavailability, and favorable penetration into soft tissues (Karchmer, 2003). Quinolones alone or in combination with other antibiotics can be one of option for treatment of mild to moderate diabetic foot infections, which are frequently mixed infections. They can be especially useful to treat combined osteomyelitis due to their ability to penetrate bone tissue. Quinolones with other antibiotics such as anti-*MRSA* and/or anti anaerobic agents also can be used for empiric treatment of complicated poly microbial skin and skin structure infection, such as poly microbial necrotizing fasciitis. However, increasing resistance, especially in *MRSA*, frequently limits the wide use of quinolones in skin and skin structure infections. Thus, quinolones should be used with caution in skin and skin structure infections (Suk *et al.*, 2014).

3.4. Mechanisms to fluoroquinolones resistance

Resistance emerged first in species in which single mutations were sufficient to cause clinically important levels of resistance (e.g., *Staphylococcus aureus and Pseudomonas aeruginosa*). Subsequently resistance has emerged in bacteria such as *Campylobacter jejuni, Escherichia coli,* and *Neisseria gonorrhoeae,* in which multiple mutations are required to generate clinically

important resistance. In these circumstances, the additional epidemiologic factors of drug use in animals and human-to-human spread appear to have contributed to resistance (Hooper, 2001).There is a worldwide emergence of fluoroquinolone resistance in *Shigella* species. Fluoroquinolone resistance in *Shigella* spp. is the end product of either a single or a combination of mutations / or efflux activity (Taneja, 2015).

Mechanisms of quinolone resistance are generally classified as three types: 1) chromosomal mutations altering the drug target enzymes to reduce drug binding, 2) chromosomal mutations that increase expression of native efflux pumps that can transport quinolones to the outside of the bacterial cell, and 3) plasmid-acquired resistance genes producing either protection of target enzymes, drug modification, or drug efflux (Suk *et al.*, 2014). The resistance mutations occur most often in a region referred to as the "quinolone-resistance-determining region (QRDR) (Hooper, 2003).Quinolone resistance mutations in the target enzymes generally occur first in the GyrA subunit of DNA gyrase in gram-negative bacteria or in the ParC subunit of topoisomerase IV in gram-positive bacteria(*Staphylococcus aureus* or *Streptococcus pneumoniae*). Less often mutations in GyrB and ParE have also contributed to resistance in clinical isolates (Casin *et al.*, 2004).

Bacteria have a number of energy dependent efflux systems in the cell membrane and envelope that can facilitate extrusion of potentially toxic agents and many of these efflux pumps have broad substrate profiles that can include quinolones. AcrAB-TolC is the major pump contributing to quinolone resistance in *E. coli.* Efflux pumps that include Quinolones among their substrates have also been associated with resistance in a number of other gram-negative bacteria, being most extensively studied in *Pseudomonas aeruginosa*. There are at least five known efflux pumps that have been shown to efflux quinolones in *P. aeruginosa*. In *S. aureus*, quinolone resistance has been associated with increased expression of NorA, NorB, and NorC pumps with both norA and norB over expression regularly found in resistant clinical isolates. Efflux also contributes to quinolone resistance in *S. pneumoniae* and *Mycobacteria.* (Suk *et al.*, 2014).

Plasmid-mediated Quinolone resistance (PMQR) was discovered in 1998 in a clinical isolate of *Klebsiella pneumoniae* that could transfer low level quinolone resistance to gram negative

bacteria. The responsible gene for PMQR was named qnr and Qnr protein was shown to bind and protect DNA gyrase and topoisomerase IV from inhibition by ciprofloxacin. Qnr itself provides only low-level resistance to quinolones, but its presence can facilitate the selection of additional resistance mutations. Other PMQR mechanisms were also identified. AAC (6')-Ib-cr confers low level ciprofloxacin resistance by acetylating of ciprofloxacin at the amino nitrogen on its piperazinyl substituent (Jacoby, 2005).

The other PMQR mechanism is plasmid-mediated quinolone efflux. Two plasmid-mediated quinolone transporters have now been found (Strahilevitz *et al.*, 2009). These PMQR determinants are increasingly being identified worldwide in clinical isolates of *Enterobacteriaceae* and in clinical and environmental *Aeromonas* species isolate (Kim *et al.*, 2009). Kim *et al* also showed that ciprofloxacin resistance increased over time, and the overall prevalence of PMQR genes tended to increase. PMQR-positive isolates had significantly higher ciprofloxacin resistance and multidrug resistance rates. The increasing frequency of ciprofloxacin resistance in *Enterobacteriaceae* was associated with an increasing prevalence of PMQR genes, and this change involved an increase in the diversity of the PMQR genes and also an increase in the prevalence of the mutations in gyrA, parC, or both in PMQR-positive strains but not PMQR-negative strains.

3.5. Clinical Occurrence of Fluoroquinolone Resistance

Fluoroquinolone resistance emerged shortly after these drugs were introduced; two species were particularly affected, *S. aureus* and *P. aeruginosa* (due to single mutation). In the case of *S. aureus* and coagulase negative *Staphylococci*, methicillin-resistant strains developed fluoroquinolone resistance more rapidly than methicillin susceptible strains (Hooper, 2000). This difference is in part explained by nosocomial transmission in some settings and by the potential for co selection with several antimicrobial agents (because of the common multidrug resistance phenotype of methicillin-resistant strains. Case-control studies have identified fluoroquinolone use as a risk factor for resistance (Pegues *et al.*, 1998).

Fluoroquinolone resistance has also increased substantially in some settings in species in which multiple mutational events are required for resistance to occur (e.g., *Campylobacter jejuni, E. coli,* and *Neisseria gonorrhoeae*). Emergence in these species would not have been predicted on

molecular grounds, suggesting that other epidemiologic factors may have come into play. For *C. jejuni*, resistance emerged in parallel in animal and human populations shortly after fluoroquinolones were introduced for use in humans and other quinolones were introduced in food animal production, particularly poultry, in parts of Europe. In the United States, where use of quinolones in food animals was introduced later, demonstrating a link between resistant *C. jejuni* strains from poultry and food products and those causing human disease was possible. Thus, for a known zoonotic pathogen such as *C. jejuni*, resistance was augmented by selection pressures in an animal reservoir of *campylobacters* (Hooper, 2001).

Fluoroquinolone resistance in *E. coli* has emerged in Europe, particularly in patients with urinary tract infections and neutropenic cancer patients with bacteremia that developed during fluoroquinolone prophylaxis (Pena *et al.*, 1995). Fecal carriage of resistant *E. coli*, however, appears to be common in both healthy adults and children in Spain (Garau *et al.*, 1999). Carriage of resistant strains by children in which fluoroquinolones were rarely used and by adults without prior quinolone exposures suggest acquisition of resistant strains were by the population at large. This occurrence suggests that acquisition of resistant strains from food sources may have resulted in substantial colonization of the human population with resistant *E. coli*, creating a reservoir of resistant organisms (Blanco *et al.*, 1997). Fluoroquinolone use in humans, which has also been shown to be a risk factor for having a resistant strain, may operate in this context to select either already fully resistant or intermediately resistant strains, accounting for the high levels of resistance and multiple mutations reported in resistant strains causing infections in humans (Hooper, 2001).

Humans are the sole reservoir for infections with *N. gonorrhoeae*. In the United States, fluoroquinolone resistance in this organism has resulted largely from clonal outbreaks caused by human to human spread (Gordon *et al.*, 1996). Newer fluoroquinolones are now incorporated into guidelines for treatment of patients with lower respiratory tract infections because of rising resistance to beta-lactams and other agents in *S. pneumoniae*, the most commonly identified bacterial pathogen in patients with community acquired pneumonia. Only recently has fluoroquinolone resistance begun to emerge in this organism, albeit at low levels. In some cases, fluoroquinolone resistant strains, like those resistant to beta lactams, have emerged because of clonal spread. Because the newest fluoroquinolones are for treating patients with respiratory tract

infections, increasing selection pressure for resistance is possible. This concern is especially great for drugs developed for use in children, who are a major reservoir of *S. pneumoniae.* Monitoring will be necessary, as will studies to indicate whether the improved therapeutic index for some fluoroquinolones can be translated into a lower risk of selection of resistant strains, either spontaneous or clonal, in the clinical setting(Hooper ,2001).

3.6. Prevalence of fluoroquinolones resistance

The emergence of fluoroquinolone resistant *S. pneumoniae* strains, although worldwide prevalence is low, is a concern to clinicians who manage respiratory tract infections. Ciprofloxacin resistant strains isolated from patients with community acquired respiratory tract infections have been reported in Spain (3.0% and 7.1%, Canada (1.7%), and the United States (1.4%). Among 2,882 *Streptococcus pneumoniae* sent to the Spanish Reference Laboratory during 2002, 75 (2.6%) were ciprofloxacin-resistant. Resistance was associated with older patients (3.9% in adults and 7.2% in patients ≥65 years of age), with isolation from noninvasive sites (4.3% vs. 1.0%).Several studies also showed that high resistance rates were observed for all antimicrobials among patients 65 years and over (Campa, 2004, Boyd *et al.*, 2008, Zec *et al.* 2016, Gururaju *et al.* 2016, Fasugba *et al*., 2016 and Mandal *et al.* 2012). Among male patients, resistance rates to most antimicrobials were high (Zec *et al.* 2016 and Boyd *et al*., 2008). A study done in Cambodia on children showed that Escherichia coli was the most common infecting isolate with high levels of resistance to most oral antibiotics(Moore *et al*.,2016)

The national use of quinolones steadily increased from 1994 to 2000 in US intensive care units (ICUs), and this use was significantly associated with decreased overall susceptibility to ciprofloxacin in the same period. The consumption of quinolones doubled during 2001-2012 in a Korean hospital with the increased ciprofloxacin resistance in clinical isolates of *Escherichia coli* in ICUs (Jun *et al.*, 2013). Another study in USA also showed fluoroquinolone resistance increased with time (Boyd *et al*., 2008).

Several investigations offer preliminary evidence that suggests that the fluoroquinolones themselves may actually predispose patients to infection with or carriage of *MRSA.* A comparison of microbiology laboratory data with antimicrobial reimbursement reports found a

significant correlation between ciprofloxacin prescriptions and the isolation of *MRSA* (Weber, 2004).

In the study for monitoring antimicrobial resistance trends, ciprofloxacin susceptibility of *E. coli* isolates from the patients having intra-abdominal infections at 37 hospital centers in North America has decreased from 84.4% to 72.2% between 2005 and 2010. For other major pathogens such as *K. pneumoniae, P. aeruginosa, Enterobacter cloacae, Klebsiella oxytoca,* and *Proteus mirabilis,* the susceptibilities for quinolones remained stable (Babinchak *et al.,* 2013). Ciprofloxacin resistance of *E. coli* isolates from community associated or hospital associated intra-abdominal infections in 2008 was 17.8% and 29.5%, respectively (Hawser *et al.,* 2010).

The quinolone resistance of *E. coli* isolates from intra-abdominal infections in Asia has been more serious with >60% ciprofloxacin resistance in *E. coli* in China. In a study, quinolone resistance of gram negative bacilli, most of which were *E. coli and K. pneumoniae,* in bacteremic intra-abdominal infections was 22.9% in Korea (Ko *et al.,* 2009). The quinolone resistance in E. coli strains in fecal flora was related to the recent quinolone use. In a Spanish study, a strong linkage between quinolone resistance in *E. coli* in human fecal flora and quinolone use in food animals, especially poultry, was also suggested. The prevalence of quinolone resistant *E. coli* in the feces of healthy persons in the community, including children who had never received quinolones, was high (24% in adults and 26% in children). Therefore, the increase of quinolone resistance in *E. coli* in intra- abdominal infections is likely the result of increasing quinolone use (Suk *et al.*, 2014). E. coli is the most common agent of urinary tract infections (UTIs) in all parts of the world (Foxman, 2002;Russo and Johnson (2003)).

Fluoroquinolone resistance varied from country to country and less so, but significantly nevertheless, from region to region. The highest regional FQR rate was seen in Latin America at 38.7%, but resistance was as high as 70% in one hospital in Panama and above 40% from three sites in Puerto Rico and Mexico. The highest fluoroquinolone resistance rates in this study were seen in India where 75% of all UTIs were non-susceptible to the fluoroquinolones. The average for the Asian countries was 33.2%. Fluoroquinolone resistance rates for Canada and the United States were 22% and 24%, respectively (Bouchillon *et al.,*2012).

The global prevalence of fluoroquinolone resistance among *Streptococcus pneumoniae* is low at around 1% .However, "hot spots" with considerably higher Fluoroquinolone resistance rates do occur (Morrissey *et al.*,2003).Quinolone resistant isolates were frequently multidrug resistant. Local resistance patterns should be considered when quinolones are prescribed for the treatment of healthcare associated pneumonia (Suk *et al.*, 2014).The emergence of antimicrobial resistance in *Streptococcus pneumoniae* worldwide is an important public health issue because this organism is the leading cause of many infections, particularly community acquired pneumonia. In recent years, the emergence of fluoroquinolone resistance is being increasingly recognized among multidrug-resistant strains of *S. pneumoniae* (Ho *et al.*, 2004).

For respiratory pathogens isolated from healthcare associated pneumonia, such as *Enterobacteriaceae, P. aeruginosa, and Acinetobacter baumannii,* the quinolone resistance rates have been higher, but with regional differences. A prospective surveillance study conducted from 2008-2009 showed a high ciprofloxacin resistance profile of *K. pneumoniae* (31.2%), *P. aeruginosa* (30.1%), and *Acinetobacter* spp. (80.7%) in Asian countries, including Korea. *Pneumoniae* for respiratory quinolones in North America remain low (<2%)(Lu *et al.*,2012).

A surveillance study for gram-negative pathogens causing UTIs in Asia-Pacific regions, showed 48.6% resistance to ciprofloxacin with wide range among different countries, from 10.0% in New Zealand to as high as 76.2% in Vietnam and 72.0% in China(Lu *et al.*,2012). A nationwide study performed in 2006 to 2007 in Korea also showed 28.4% Ciprofloxacin resistance for *E. coli* isolates causing community onset UTIs with dissemination of epidemic and virulent ciprofloxacin resistant *E. coli* clones (Lee *et al.,* 2010). In prospective Korean nationwide surveillance during 2010 to 2012, the ciprofloxacin resistance in *E. coli* isolates from women having community-acquired acute pyelonephritis was 20.0% (Kim *et al.*, 2014). Another multicenter study in 2012 also showed similar (22.5%) ciprofloxacin resistance in *E. coli* isolates from Korean women having community associated acute pyelonephritis (Park *et al.*, 2014). Consequently, the choice of empiric treatments for UTIs has now become challenging, since 20–50% of E. coli isolates are now resistant to the first-line of antibiotics (Sanchez etal.,2012)

A study in Nigeria showed that pathogen's susceptibility to agents varied but the activities of ciprofloxacin and nalidixic acid against *E. coli* recorded significant yearly decrease. The overall susceptibilities of *E. coli* were 58%, 34% and 15% for ciprofloxacin, norfloxacin and nalidixic acid respectively. Multi fluoroquinolones resistant cases (282, 43.6%) were observed in *E. coli* which increased from 30.2% in 2006 to 57.5% in 2009. The study observed a rapid and progressive loss of activities of quinolones; increasing multi fluoroquinolone resistant, high resistance rates and poor inter activities relations between the fluoroquinolones against *E. coli* in the region (Ohieku *et al.*, 2013).

Another study conducted in Iran showed resistance for nalidixic acid, ciprofloxacin and norfloxacin were 42.3%, 28.2% and 48.5% respectively (Akya *et al*., 2015). A retrospective analysis in tertiary care hospital in southern India showed that the overall resistance pattern of antibiotics to uropathogens was the highest to nalidixic acid (79%).Nalidixic acid and first generation fluoroquinolones have limited value for the treatment of UTI (Somashekara *et al*., 2014)

In a population based survey of 3,996 persons in Indonesia, fluoroquinolone resistant *Escherichia coli* was prevalent in the fecal flora of 6% of patients at hospital admission and 23% of patients at discharge, but not among healthy relatives or patients visiting primary healthcare centers 2% virulence traits are present . Fluoroquinolone resistant *E. coli* has been reported increasingly during the last decade in both the hospital environment and the community. Moreover, fluoroquinolone resistant *E. coli* strains often show resistance to other drugs, such as ampicillin, tetracycline, chloramphenicol, trimethoprim sulfamethoxazole, and gentamicin (Kuntaman *et al.*,2005).A study in Brooklyn, New York revealed that susceptibility rates to ciprofloxacin decreased dramatically from 1997 to 1999 (47% to 16%)(Quale, 2002). A trend analysis done in Australian hospitals from 2009 to 2013 showed that significant increases in bacterial resistance to ciprofloxacin over five years (Fasugba *et al*., 2016). A three year retrospective study by Sharma *et al*., 2016 showed on an average over the three years E. coli showed high amount of resistance to fluoroquinolones (75 %). There were consistent linear increases in resistance to ciprofloxacin and norfloxacin, both fluoroquinolones. Both ciprofloxacin and norfloxacin exhibited significant positive correlations between the resistance of samples and the year the samples (Rodrigues *et al*., 2016)

3.7. Factors Leading to Acquired Bacterial Resistance to Antibiotics

Misuse of Antibiotics by health professionals in Clinical Practice

Antibiotic use provides selective pressure favoring resistant bacterial strains; inappropriate use increases the risk for selection and dissemination of antibiotic resistant bacteria. Maslow *et al.*, 2005 showed that prior FQ use was the only independent risk factor for FQ-resistant *E. coli* carriage. Under certain circumstances, resistance to fluoroquinolones can emerge during treatment. Some studies have reported that < 50% of all patients taking quinolones for prostatitis are colonized with quinolone resistant *Escherichia coli* strains and have described quinolone resistance after treatment courses of as few as 3 days. Although other factors are likely to contribute to resistance in persons, ecologic data show an association between fluoroquinolone use and resistance (Cheng *et al.*, 2012).

Suk *et al*.,2014 indicated that the known risk factors for quinolone resistance in uropathogenic *E. coli* isolated from community-onset acute pyelonephritis are prior exposure to quinolones, previous hospitalization, recurrent UTIs, previous invasive procedures, the presence of complicated UTIs, chronic diseases including neurologic diseases, age over 50 years, and presence of a urinary catheter in the past 6 months. Another concern caused by quinolone resistance is its high association with extended spectrum beta-lactamase (ESBL) production in *Enterobacteriaceae*. The interplay between prior heavy antibiotic use and conditions favoring patient-to-patient transfer of multidrug-resistant organisms or the occurrence of transferable plasmids carrying genes conferring resistance to quinolones and other antimicrobials could be contributing factors. The choice of appropriate antibiotics can be very limited in quinolone resistant, ESBL producing uropathogens because of their multidrug resistant nature. The known risk factors for infection or colonization by Levofloxacin resistant *S. pneumoniae* are previous exposure to quinolones, healthcare associated infection, and residence in a nursing home, presence of chronic obstructive pulmonary disease, and presence of cerebrovascular disease (Jiménez *et al.,* 2005).

The unnecessary prescription of antibiotics seen in industrialized nations has also been documented in many developing countries, particularly in cases of acute infantile diarrhea and viral respiratory infections. A study done in Mekelle general hospital showed that antibiotics

were prescribed irrationally and prescriptions containing one or more antibiotics constituted 73.7% of all prescriptions and around 5.9% of encounters were treated without any diagnosis (Sebsibie *et al.*, 2014). Clinical misuse of antibiotics may be more common among private practitioners than among public health personnel. The demand for antibiotics seen in private patients is higher, (Paredes *et al.*, 1996).

Drug labels and package inserts often fail to provide accurate information and in less industrialized countries, patients often pressure physicians to prescribe antibiotics (Okeke *et al.*, 1999). Another contributing factor for bacterial resistance of antibiotics is OTC sell of antibiotics. A recent study done by Gebretekle *et al.* in Ethiopia indicated that the nonprescription sales of antibiotics were common for drug like ciprofloxacin. The poor, less educated and younger groups of the population were reported to frequently request antibiotics without prescription. The main reasons for nonprescription sale of antibiotics by pharmacy professionals were found to be related to pharmacy owner's influence to maximize revenue, customer's pressure, weak regulatory mechanism and professional conflicts of interest (Gebretekle *et al.* 2016).

Contribution of other than human use fluoroquinolone to fluoroquinolone resistance

E. coli is widely distributed among humans, animals, water, and some foods; thus, selective pressure is likely to be exerted by antimicrobial drug use in human and agricultural sectors. This likelihood is supported by molecular typing studies in which researchers examined *E. coli* strains resistant to trimethoprim-sulfamethoxazole, quinolones, and extended-spectrum cephalosporins in humans and in commercial poultry products in the United States, where these antimicrobial drugs are or have been used in poultry production (Johnson *et al.*, 2007).

Misuse of Antibiotics by Unskilled Practitioners and by the Public

In many developing countries, well-trained health personnel are scarce and cannot serve the entire population, especially in rural areas. Community health workers and others with minimal training treat minor ailments. Unqualified drug sellers offer alternative drugs when the prescribed drugs are out of stock or refill prescriptions without consulting the prescriber. A high proportion of patients in some developing countries are treated by untrained practitioners (Okeke *et al.*, 1999).Antibiotics can be purchased without prescription in most developing countries.

Cheng *et al*. showed that resistance in *N. gonorrhoeae* and *S. typhi* are also influenced by variations in global epidemiology of disease and in ease of availability of quinolone antimicrobial drugs, including over-the-counter access in Asia, where much higher levels of resistance have been documented .In many African, Asian, and Latin American countries, antibiotics are readily available on demand from hospitals, pharmacies, patent medicine stalls (drugstores), road side stalls, and hawkers. Alternate sources offer the option of purchasing small quantities of medicines, while hospitals require purchase of the complete 5 or 7 day antibiotic regimen. Another cause of antibiotic abuse and selection for resistant bacteria is poor patient compliance.

Poor Quality, Expired, Counterfeit and Adulterated Antibiotics

Degradation products or adulterants in poor quality antibiotics can produce sub inhibitory concentrations in vivo, which increase the selection of resistant strains. Therapeutic failure is the only indication of substandard drugs. Analytic laboratories to detect substandard drugs are uncommon, and when they exist, health workers, distributors, and consumers are often unaware of them. Some pharmacologically active drugs produced in industrialized countries have expired when distributed in developing countries. Expired drugs may receive new labels, be dumped without a label change, or be donated rather than sold. Tax deductions and the cost of liquidation are incentives for donating expired or near-expired drugs. Some drugs may also be sold in developing countries do not contain the concentration of active substances on their labels, even at the time of manufacture .Because of the profusion of generic drugs in developing countries, a substantial proportion of counterfeit drugs go undetected. Herbal preparations in developing countries are often adulterated with orthodox medicaments. Although the adulteration of such products with antibiotics has not been reported, such practices may be common. (Okeke *et al*., 1999).

Rapid urbanization

When a population grows beyond its resources, overcrowding and poor hygiene and sanitation result. Poor hygiene and sanitation increase disease prevalence. Isolates from E. coli strains in urban Nigeria (Lagos) showed significantly more antimicrobial resistance than ones in rural/suburban Nigeria (Byarugaba, 2004)

Pollution and environmental degradation

Contamination can occur from leaking septic tanks, run-off of manure from fields and feces in the water brings human or animal pathogens in contact with potentially resistant bacteria. This water is used for drinking or irrigation for agriculture. Increased levels of ozone air pollution leads to more micro-organisms enter drinking water from associated increase in rainfall and run-off hence higher risk for resistance transfer (Dolliver, 2008).

Demographic changes

A frequently overlooked social factor that can exacerbate AMR is the effect of a high concentration of very young and old patients in a health care system. Since it is easier for infection to take root in these populations, the effects of the shifting patient population on the spread of infection should be examined.

AIDS epidemic

The AIDS epidemic is a strong risk factor for the formation of AMR. Because HIV diminishes a body's ability to fight infections; many people living with HIV/AIDS frequently use antimicrobials prophylactically and to treat infections. Indeed, it is likely that HIV is impacting the state of AMR (Byarugaba, 2004).

Growth of global trade and travel

Globalization has enabled microbes to travel fast and far, leaving no region unaffected. AMR is more pronounced in developing countries. AMR commensal organisms are often part of the normal gut flora in developing country residents. Urban migration, inadequate sewage disposal and overcrowding encourage AMR dissemination (Memish *et al*., 2003)

Role of poverty, Animal feed, Antibacterial Cleaning Products are also the contributing factors for antimicrobial resistance. (Aiello, 2005 and Byarugaba, 2004).

3.8. Antimicrobial resistance containment mechanism

1. Reduce the need for antibiotics through improved water, sanitation, and immunization. Improving coverage for existing vaccines and adding new ones, improving access to clean water

and sewerage systems, and ensuring a safe and healthful food supply all reduce the need for antibiotics, thereby reducing antibiotic resistance rates.

2. Improve hospital infection control and antibiotic stewardship.

Better hygiene, particularly hand washing with soap or using alcohol disinfectant between patients, and antibiotic stewardship programs reduce infection rates. Surveillance of resistance and hospital-acquired infections gives administrators information for management and policy decisions.

3. Change incentives that encourage antibiotic overuse and misuse to incentives that encourage antibiotic stewardship. Eliminating economic incentives that encourage the overuse of antibiotics all along the supply chain in hospitals, in communities, and in agriculture can conserve antibiotic effectiveness.

4. Reduce and eventually phase out antibiotic use in agriculture. Eliminating antibiotic use for growth promotion and minimizing use for disease prophylaxis need not jeopardize animal or human health.

5. Educate and inform health professionals, policymakers, and the public on sustainable antibiotic use. Education and guidelines for healthcare professionals, engagement with policymakers, and national awareness campaigns for the public will begin changing the norms in antibiotic use and promote conservation.

6. Ensure political commitment to meet the threat of antibiotic resistance. Presenting the case to policymakers and gaining their political and financial support are critical to success.

During the course of the article selection stage for the literature review, the search on search engines in the internet of the terms “Assessment of Bacterial resistance trends; susceptibility contributing factors to fluoroquinolones in Ethiopia” did not deliver current articles to be incorporated to the literature review. This showed the researcher that the gap that could be studied. Therefore this study aimed to assess the current trend of bacterial resistance trends to fluoroquinolone.

4. Objectives

4.1. General Objective

- To assess the bacterial resistance trend to fluoroquinolone among patients' specimens analyzed at International Clinical Laboratories and contributing factors in Addis Ababa, Ethiopia

4.2. Specific Objectives

- To estimate the prevalence of bacterial isolates resistance to common fluoroquinolones
- To assess resistance trends of bacterial isolates to fluoroquinolone over time.
- To explore the contributing factors for bacterial resistance to fluoroquinolones from the perspective of prescribers and dispensers in Addis Ababa, Ethiopia.

5. Method

5.1. Study setting and period

The study was conducted in an international clinical laboratory (ICL), a private owned company in Ethiopia. Provision of services was started in 2004 with the aim of providing quality laboratory services all over Ethiopia. Based on this target, ICL's service is now expanded throughout Addis Ababa and the different regions including Bahir Dar, Mekelle, Hawassa, Adama, Gondar, Jimma and Harrar. The main laboratory is located in Kirkos sub city of Addis Ababa city administration. It has 8 Patient Service Centers (PSC's) and 1 Satellite Laboratory. Patients get referred to this laboratory from both private and government owned health facilities located in all over the country. As a part of commitment to quality, ICL participates in international quality assurance program with American Proficiency Institutes three times a year. International clinical laboratory is the first and the only laboratory in Africa accredited by Joint commission International - USA in 2004 and 2007.With its high tech laboratory, ICL has the following departments: Hematology, Serology, Chemistry, Immunology, Microbiology and Molecular diagnostics, Surgical pathology and Cytopathology and Referrals section.

5.2. Study design

A retrospective, cross-sectional study was conducted from September 2016 to December 2016. In addition, a phenomenological qualitative method using an in-depth interview was employed to identify the contributing factors from the perspective of prescribers and dispensers.

5.3. Source and study population

The source population for this study was patients from all over Ethiopia whose samples were sent to microbiology laboratory in the ICL. The study population was all samples from patients which were tested for fluoroquinolone resistance from January 2013 and December 2016.

The source and study population for qualitative part of this study was all prescribers and dispensers working in health facilities of Addis Ababa.

The study population for qualitative part of was selected prescribers and dispensers working in health facilities of Addis Ababa

5.4. Eligibility criteria

Those data on registers with complete record of the susceptibility test and whose isolates were tested for fluoroquinolones resistance were included in the study.

5.5. Sampling and Sample size determination

All data registered from January 2013 to December 2016 was included in the study by using conventional sampling technique. For the qualitative study, the key informants were selected purposively. Data collection was terminated once no new information around the four predefined theme was obtained. There were 10 interviews with key informant pharmacy professionals and 10 interviews with key informant Clinicians.

5.6. Data collection instrument and management

Data collection instrument

A data abstraction format was used to collect patients address and laboratory data such as, specimen type, susceptibility test results and year of test (Annex-I). For the qualitative data, the interview involved the use of open-ended interview guide with flexible probing techniques that would help us to investigate factors contributing for Fluoroquinolones resistance from prescribers' and dispensers' perspective (Annex –II). The interview questions were classified into two sections. The first section includes information on age, sex, highest educational level attained and professional work experience of pharmacy professionals and physicians. The second section includes 8 predefined questions with probing questions to explore the professional's view on the current trends of prescribing and dispensing antimicrobials, their resistance, contributing factors and measures that should be taken to contain antimicrobial resistance in general and fluoroquinolones resistance in particular.

Quality control

Data collectors who were laboratory technician were trained on how to collect the data using data abstraction format and the quality of the data to be collected; the structured data abstraction format and the interview guide were pretested and modifications were made accordingly. During the data collection, there was a regular supervision and the collected data was checked for error and completeness.

For the qualitative data, the principal investigator carried out all the interviews and all interviews were tape recorded. Audio-recorded interviews were translated and transcribed by the principal investigator. Furthermore, findings of the study were communicated to some of the study participants for authenticity of interpretations.

5.7. Description of study variables

Dependent variable

Resistance to fluoroquinolones, trends of resistance to fluoroquinolones

Independent variable

Age, Sex, type of specimens, region

Year of specimen collection

Type of bacterial strains (isolates)

5.8. Data entry and analysis

The quantitative data was entered and analyzed using SPSS version 16. Simple descriptive analysis such as percentage, proportion and mean, was computed. Tables, charts and graphs were used to present summary results. Also inferential statistics was conducted using logistic regressions to explore possible relationship between independent and dependent variables. In addition chi-square for trend was used to analyze the resistance across the years. The qualitative data analysis involved an intensive hearing and reading in order to identify key themes. The Amharic version of the interview was translated to English coded and categorized into main themes manually. A thematic analysis was used to analyze the data.

5.9. Ethical consideration

Ethical approval was obtained from the School of Pharmacy, Addis Ababa University and permission was secured from International Clinical Laboratories for their record and participants' health institutions. Informed verbal consent was obtained from study participants, following an explanation about the purpose of the study and what to expect from them. Finally, the participants were assured about confidentiality and anonymity of the information by assuring that the information they gave would not be accessed to anybody other than the investigator and any information that they gave was kept confidential. Also their names and personal identifiers

would not be indicated. In reporting of the qualitative findings, codes were used to maintain anonymity of participants.

5.10. Operational definitions

Body Fluid: Any sterile fluid except cerebrospinal fluid (ICL definition) like synovial fluid and pleural fluid.

Discharge: In this study includes any fluids that are taken from both eye and ear.

5.11. Method of antimicrobial susceptibility testing

The method used for testing was disc diffusion method from 2013 to 2015 and BD phoenix second generation microbiology system in 2016. A report of "Susceptible" indicates that the pathogen is likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable. A report of "Intermediate" indicates that the result should be considered equivocal, and, if the microorganism is not fully susceptible to alternative, clinically feasible drugs, the test should be repeated. This category implies possible clinical applicability in body sites where the drug is physiologically concentrated or in situations where high dosage of drug can be used. This category also provides a buffer zone which prevents small uncontrolled technical factors from causing major discrepancies in interpretation. A report of "Resistant" indicates that the pathogen is not likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable; other therapy should be selected.

6. Results

6.1. Patients' demographic information and source of specimen

A total of 7,889 isolates were identified from 4310 patients at the International Clinical Laboratories main branch in Addis Ababa. Of these, majority 2630(62.2%) of the patients were female patients. Age of patients ranged from 1 day to 95 years with a mean age of 42.2± 22.5 years. The highest proportion 1138 (26.4 %) of the patients were 60 years or older followed by 30-44 years old patients, accounting 987 (22.9%). Most the analyzed samples were from Addis Ababa 4046 (94.3%) and the rest were from other regions of Ethiopia where ICL branches are available (Table 1).

Table 1: Sex and age distribution of patients in ICL from 2013-2016.

Characteristics	Number	Percentage
Gender		
Male	1630	37.8
Female	2680	62.2
Age		
<15 years	509	11.8
15-29 years	840	19.5
30-44 years	987	22.9
45-59 years	836	19.4
60 and above years	1138	26.4
Region		
Addis Ababa	4046	94.3
Others*	264	5.7

*Others include Amhara, Oromia, Harar, Southern nations and nationalities

As depicted in Table 2, *E.coli, Klebsiella, Staphylococcus* and *Streptococcus* isolates were more prevalent in females than males. On the other hand, *Enterobacter, Pseudomonas* and *Enterococci* were more prevalent in males than females. The highest groups of bacterial isolates (26.4%) were obtained from the age group greater than or equal to sixty years. The second higher bacterial isolates (22.9%) were obtained from age group of thirty to forty four years. In all groups *E.coli* from gram negative bacteria and *Staphylococcus* from gram positive were the two major isolates identified. *Enterococcus* isolates were the lowest isolates in all age groups.

Table 2: Pattern of bacterial isolates with age and sex in ICL 2013-2016.

Variable		*E.coli* N(%)	*Enterobator* N (%)	*Enterococci* N (%)	*Klebsiella* N (%)	*Pseudomonas* N (%)	*Staphylococcus* N(%)	*Streptococcus* N(%)	Others* N (%)	Total
Sex	Male	1365(30.2)	114(60.6)	52(57.1)	237(41)	176(59.96)	740(46.7)	46(44.2)	253(47.5)	2983(37.8)
	Female	3152(69.8)	74(39.4)	39(42.9)	341(59)	118(40.1)	844(53.3)	58(55.8)	280(52.5)	4906(62.2)
Age	<15 years	422(9.3)	37(19.7)	10(11)	58(10)	53(18)	225(14.2)	12(11.5)	111(20.8)	928(11.8)
	15-29 years	863(19.1)	39(20.7)	11(12.1)	71(12.3)	57(19.4)	383(24.2)	23(22.1)	95(17.8)	1542(19.5)
	30-44 years	1039(23)	28(14.9)	13(14.3)	139(24)	51(17.3)	403(25.4)	34(32.7)	103(19.3)	1810(22.9)
	45-59 years	925(20.5)	31(16.5)	9(9.9)	125(21.6)	57(19.4)	266(16.8)	12(11.5)	104(19.5)	1529(19.4)
	60 years and above	1268(28.1)	53(28.2)	48(52.7)	185(32)	76(25.9)	307(19.4)	23(22.1)	120(22.5)	2080(26.4)

*Others include: *Citrobacter*, *Acinobactor*, *Morganella*, *Proteus*, *Shigella*, *Haemophilus*, ,*Barklolderia*, *Kluyvera*, *Providentia* and *Serratia*

6.2. Commonly identified bacterial isolates

Table 3 shows the commonly isolated bacterial isolates. Over the four year retrospective record review period, the number of bacteria observed in ICL was fluctuating over time. The number of isolates, however, decreased in 2015 to 1889 (23.9%) and then increased to 2079 (26.4%) in 2016. From the two major bacteria isolated; *E.coli* showed increase in number whereas isolated *Staphylococcus* showed decrease in number across the four year.

Table 3: Distribution of bacterial isolates across years in ICL 2013-2016

Year	*E.coli*	*Entero bacter*	*Enterococci*	*Klebsiella*	*Pseudom onas*	*Staphyloc occus*	*Strepto coccus*	Others*	Total
2013	909	12	0	44	32	848	34	79	1958
2014	1148	53	28	141	101	327	13	152	1963
2015	1162	41	41	219	94	257	12	63	1889
2016	1298	82	22	174	67	152	45	239	2079
Total	4517	188	91	578	294	1584	104	533	7889

*Others include: *Citrobacter, Acinobactor, Morganella, Proteus, Shigella, Haemophilus, ,Barklolderia, Kluyvera, Providentia and Serratia*

Out of the total isolates, *E.coli* takes the majority 4517 (57.2%) of the isolate followed by *Staphylococcus* which accounted 1584 (20.1%). The highest proportion 6238(79.1%) of the isolates were identified from urine followed by wound 781(9.9%).

Table 4:: Distribution of bacterial isolates within specimen types in ICL 2013-2016.

Specimen type	*E.coli* N (%)	*Enterob acter* N (%)	*Enteroc occi* N (%)	*Klebsiel la* N (%)	*Pseudo monas* N (%)	*Staphyloc occus* N (%)	*Strepto coccus* N (%)	Others* N (%)	Total
Blood	47(28.5)	3(1.8)	0(0)	16(9.7)	15(9.1)	72(43.6)	3(1.8)	9(5.6)	165
Body fluid	39(30)	0(0)	2(1.8)	1(0.8)	17(13.1)	43(33.1)	6(4.6)	22(16.9)	130
CSF	27(26.2)	8(7.8)	0(0)	8(7.8)	0(0)	29(28.2)	3(2.9)	28(27.2)	103
Discharge	46(14.8)	7(2.3)	0(0)	19(6.1)	37(11.9)	151(48.7)	6(1.9)	44(14.2)	310
Sputum	0(0)	0(0)	0(0)	27(32.9)	10(12.2)	34(41.5)	2(2.4)	9(11)	82
Stool	2(2.5)	0(0)	0(0)	0(0)	4(5)	2(2.5)	56.2()	67(83.8)	80
Urine	4207(67.4)	148(2.4)	89(1.4)	440(7.1)	145(2.3)	844(13.5)	70(1.1)	295(4.7)	6238
Wound	149(19.1)	22(2.8)	0(0)	67(8.6)	66(8.5)	409(52.4)	9(1.2)	59(7.6)	781

*Others include: *Citrobacter*, *Acinobactor*, *Morganella*, *Proteus*, *Shigella*, *Haemophilus*, *Barklolderia*, *Kluyvera*, *Providentia* and *Serratia*.

6.3. Fluoroquinolones susceptibility

Out of the total 7889 isolates, 3818 (48.4%) of them were sensitive, 3565 (45.2%) were resistant and 506 (6.4%) were intermediate. Table 4 shows that resistance was high for nalidixic acid 394 (63.3%) followed by norfloxacin 1613 (44.4%). Out of the total 3637 cases for ciprofloxacin, 1789 (49.2%) were sensitive, 1558 (42.8%) were resistant and 290 (8.0%) were intermediate. Generally resistance was highest for nalidixic acid 394 (63.3%) followed by norfloxacin 1613(44.4%) and then ciprofloxacin 1558 (42.8%) (Figure 1).

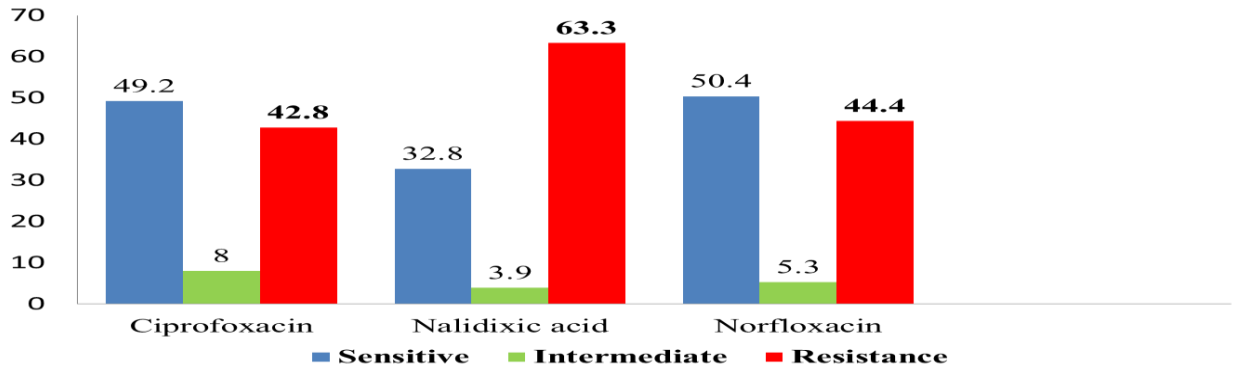

Figure 1: Fluoroquinolones susceptibility pattern in ICL 2013-2016.

Table 5 shows distribution of bacterial isolate to fluoroquinolone resistance. There was wide variability among bacterial isolate to fluoroquinolone resistance. *Enterococci* and *E.coli* isolates developed higher resistance to ciprofloxacin and norfloxacin. The resistances of *Enterococci* and *E.coli* isolates to ciprofloxacin were 26(54.2%) and 949(47.4%), respectively. Compared to others isolates, *Pseudomonas* showed less resistance to ciprofloxacin. Resistance to nalidixic acid was found to be high by most of the pathogens; the highest being by *Enterococci* isolates 7(77.8%) followed by *Pseudomonas* isolates 15(75.0%).

Table 5: Resistance pattern of bacterial isolates to fluoroquinolones in ICL 2013-2016.

Isolates	Fluoroquinolone resistance		
	Ciprofloxacin N (%)	Norfloxacin N (%)	Nalidixic acid N (%)
E.coli	949(47.4)	1007(48)	272(65.4)
Enterobacter	38(42.7)	35(44.3)	14(70.0)
Enterococci	26(54.2)	19(55.9)	7(77.8)
Klebsiella	94(35.1)	91(35.8)	25(44.6)
Pseudomonas	36(27.3)	41(28.9)	15(75.0)
Staphylococcus	310(39.1)	319(42.3)	26(68.4)
Others*	105(34.2)	101(37.8)	35(55.6)
Total	1,558(42.8)	1,613(44.4)	394(63.3)

*Others include: *Citrobacter, Acinobactor, Morganella, Proteus, Shigella, streptococcus, Haemophilus, Barklolderia, Kluyvera, Providentia* and *Serratia*.

Resistance to ciprofloxacin 1200(43.9%) and norfloxacin 1264(44.9%) were relatively higher for gram negative isolates, whereas higher resistance against nalidixic acid 41(69.5%) was shown by gram positive isolates (Table 6).

Table 6: Pattern of susceptibility of gram positive and gram negative bacteria to fluoroquinolones in ICL 2013-2016.

Type of drug	Isolate category	Sensitive N (%)	Intermediate N (%)	Resistant N (%)	Total
Ciprofloxacin	Gram-ve	1359(49.7)	175(6.4)	1200(43.9)	2734(100)
	Gram +ve	430(47.6)	115(12.7)	358(39.6)	903(100)
Nalidixic acid	Gram -ve	186(33.0)	24(4.3)	353(62.7)	563(100)
	Gram +ve	18(30.5)	0(0.0)	41(69.5)	59(100)
Norfloxacin	Gram -ve	1431(50.9)	118(4.2)	1264(44.9)	2813(100)
	Gram +ve	394(48.2)	74(9.1)	349(42.7)	817(100)

Sensitivity to fluoroquinolones varies across sample type, ranging from the highest in stool 94(92.5%) to the lowest in urine 2752(44.1%). The resistance against fluoroquinolones was the highest in sputum 41(50.0%) and the lowest in stool samples 5(6.2%). Isolates from urine were

3110(49.9%) resistant, 376(6.0%) intermediate and 2752(44.1%) sensitive. Samples from wound were 68(8.7%) intermediate, 207(26.5%) resistant and 506 (64.8%) sensitive (Figure 2).

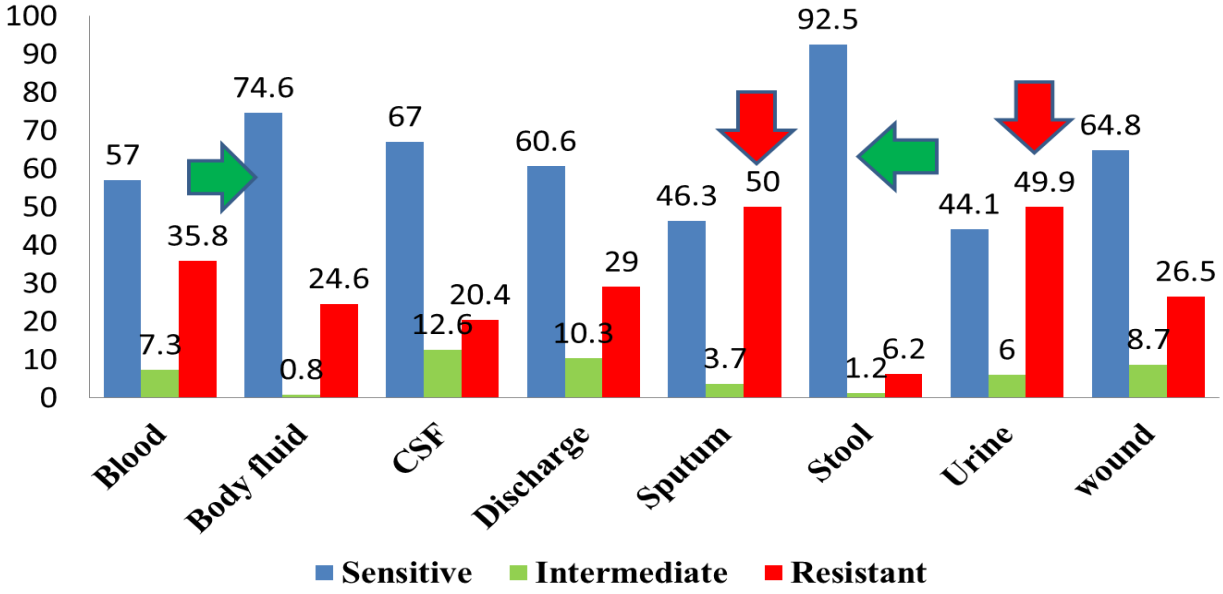

Figure 2: Fluoroquinolones response by specimen type in ICL 2013-2016.

Resistances to fluoroquinolones were more common in males than females. Fluoroquinolones were resistant, intermediate and sensitive in 1402(47.0%), 203(6.8%) and 1378(46.2%) male cases, respectively. Similarly, fluoroquinolones were resistant, intermediate and sensitive in 2163(44.1%) 303(6.2%) and 2444(49.7%) female cases, respectively (Table 7).

Table 7: Pattern of fluoroquinolones response across sex in ICL 2013-2016.

Sex of the patient	Sensitive N (%)	Intermediate N (%)	Resistant N (%)	Total
Male	1378(46.2)	203(6.8)	1402(47.0)	2983(100)
Female	2440(49.7)	303(6.2)	2163(44.1)	4906(100)
Total	3818(48.4)	506(6.4)	3565(45.2)	7889(100)

As age of patients increased sensitivity to fluoroquinolones decreases. The sensitivity to fluoroquinolones was as high as 642(69.2%) in those less than 15 year old and as low as 635(30.5%) in those patients 60 years and above age category. In general, resistance to fluoroquinolone increases as patients' age increases ranging from 212(22.8%) in age category less than 15 years to 1298(62.4%) in age 60 years and above. That means the resistance to

fluoroquinolones nearly triples between the age less than 15 years compared to age category 60 years and greater (Figure 3).

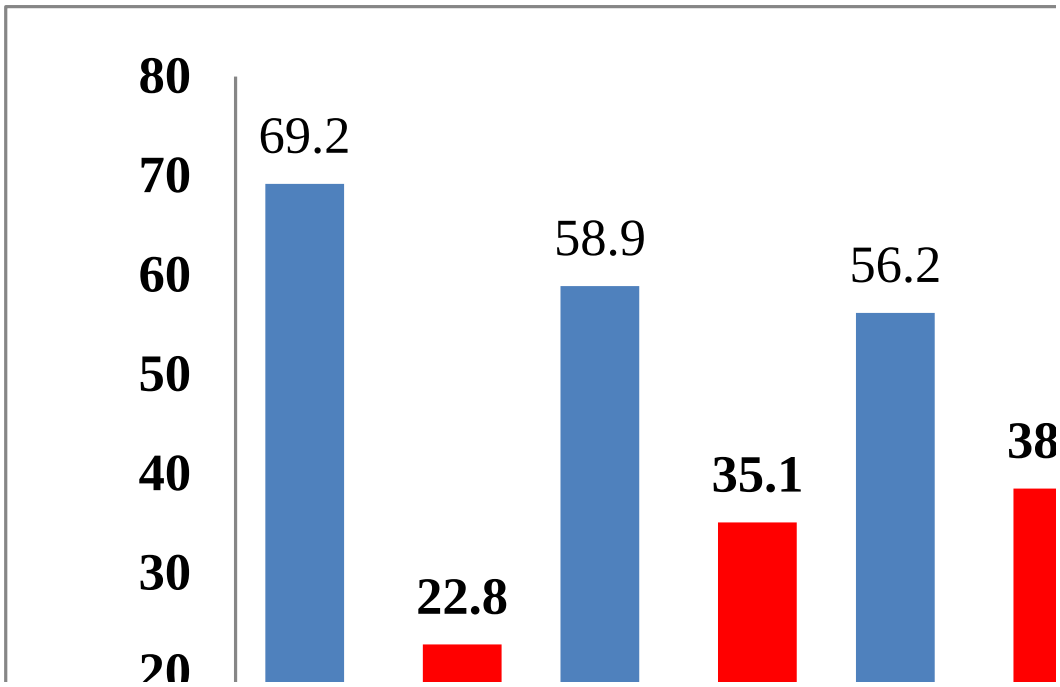

Figure 3: Pattern of fluoroquinolones response across age groups in ICL 2013-2016.

6.4. Trends of bacterial resistance to fluoroquinolones

In general, in over the four year period bacterial resistance to fluoroquinolones increased by 16% from 40% in year 2013 to 46% in year 2016. That means the resistance was increasing on average 4% every year. However, with in the four year period the resistance was the highest in year 2015 (Figure 4).

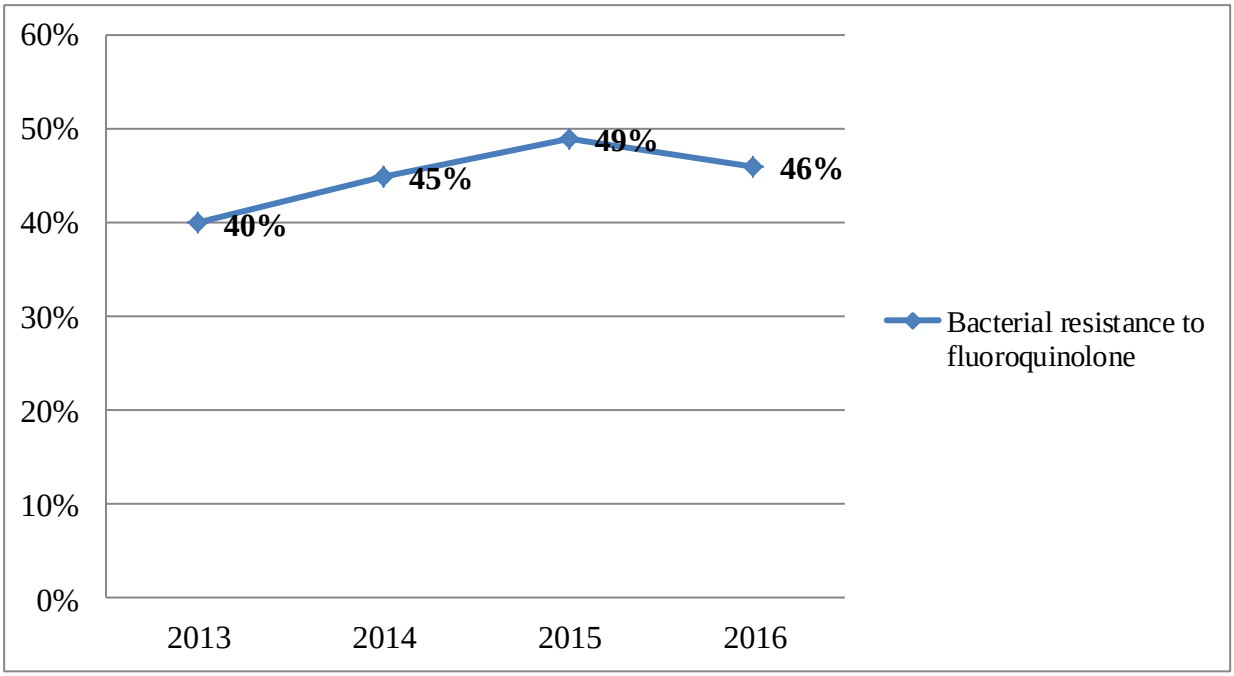

Figure 4: Trends of resistance to fluoroquinolones over the four year in ICL 2013-2016.

The Chi square for trends analysis also showed that there is significant association between resistance against fluoroquinolones and years of diagnosis (P value= 0.00002; Chi-square for linear trends (extended mantel haenszel) =17.843) (Table 8).

Table 8 : Chi square for trend analysis of bacterial resistance against fluoroquinolones over the four year in ICL 2013-2016.

Year of diagnosis	Resistant	Sensitive and intermediate	Odds ratio
2013	791	1167	1.00
2014	886	1077	1.214
2015	926	963	1.419
2016	962	1117	1.271

Figure 5 shows the trend of drug resistance over four years by type of fluoroquinolones from year 2013 to year 2016. The highest increase in resistance trend was observed against ciprofloxacin. Ciprofloxacin resistance showed an increment of 15%. The trend for resistance to

ciprofloxacin was increased for three years 39%, 43% to 46% in year 2013, 2014 and 2015 and then decline to 45% in year 2016.

The resistance against norfloxacin has continuously increased between year 2013, 2014 and 2015 from 42% to 45% and 46%, respectively. Then the resistance decreased to 45% in year 2016. Data for nalidixic acid was not obtained for the year 2013 because the diagnostic test had not been performed in the indicated year in ICL. However, the resistance to nalidixic acid increased from 61% in 2014 to 63% in 2015 and then to 67% in year 2016(Figure 5).

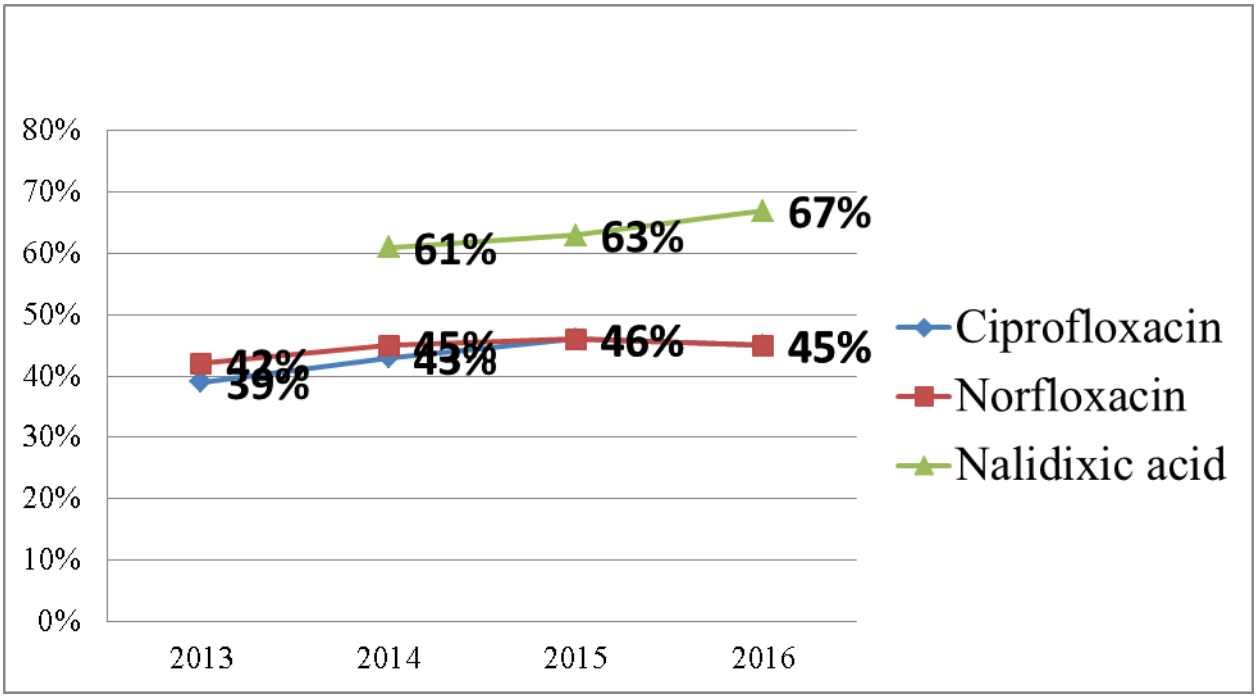

Figure 5: Trend of bacterial resistance by type of fluoroquinolones over the four years in ICL 2013-2016.

In general the drug resistance was increasing over four year period in male population; while it showed slight increase from 43% to 46% (2013-2015) at similar level for female population. The drug resistance among males was increased form 37% in year 2013 to 55% to year 2015 and declined to 51% in year 2016. Hence fluoroquinolone resistance was increased by 14 percentage point. The patterns of resistance among males and females are similar except the fact that the extent of increment in resistance is low among females as compared to males. The drug resistance among females continuously increased in years 2013, 2014, 2015 from 43%, to 45% and 46%. Then the drug resistance declined back to the year 2013 level of 43% in year 2016(Figure 6).

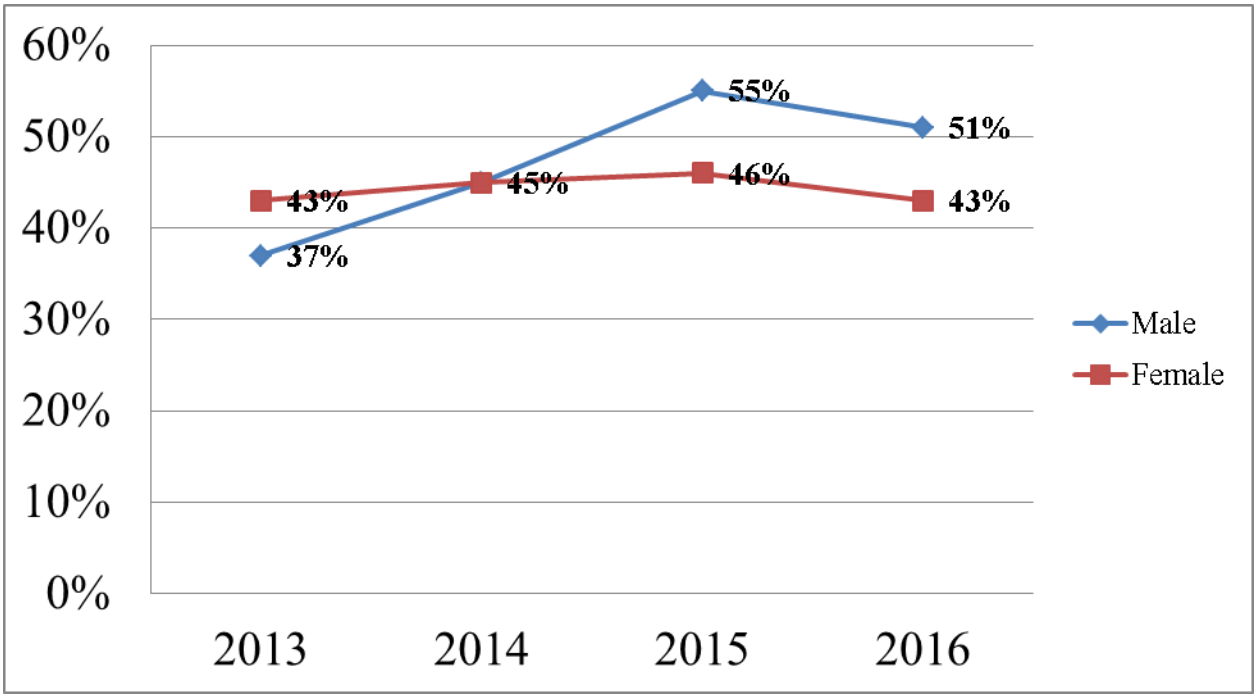

Figure 6: Trend of bacterial resistance by sex over the four year in ICL 2013-2016.

Figure 7 shows the trends of fluoroquinolones resistance by isolate type is shown in Figure 5. In general there was an increasing trend of fluoroquinolones resistance among gram negative bacteria species; while there was a decreasing trend for gram positive bacterial species. Resistance to fluoroquinolones increased by gram negative bacteria species from 34% in year 2013 to 46% in year 2014 and then to 52% in year 2015. It declined from 52% in year 2015 to 48% in year 2016. On the other hand, resistance of gram positive bacterial species to fluoroquinolones decreased over the four years period of 2013, 2014, 2015 and 2016 from 48%, 41%, 34% and 31%, respectively.

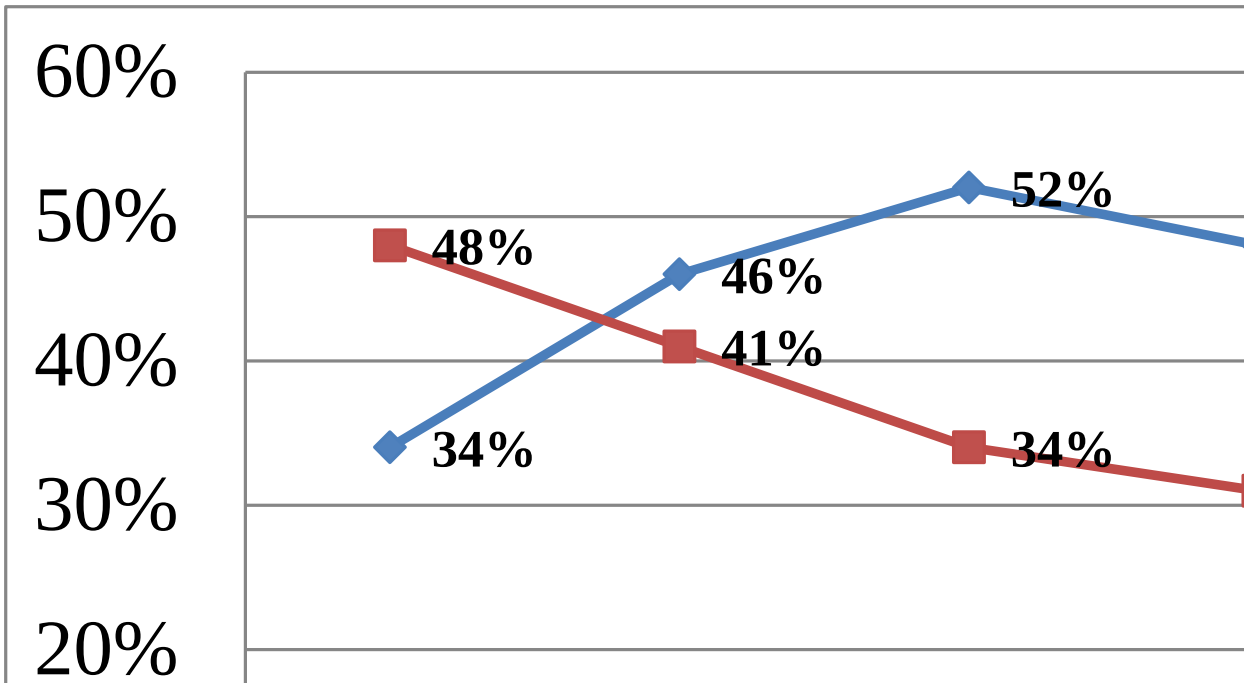

Figure 7: Trend of resistance by gram negative and gram positive bacteria over the four year in ICL 2013-2016.

6.5. Factors associated with resistance to fluoroquinolones

The findings show that age and year showed significant association with sensitivity to fluoroquinolones. As age of patients increased; resistance to fluoroquinolones increased. For instance, resistance to fluoroquinolones among patients of age 60 and above years was more than 5 times {AOR= 5.63 (4.71, 6.73)} that of among patient's age less than 15 years. In age group 30 to 44 years resistance was twice more than in patient's age less than 15 years AOR =2.07 (1.72, 2.48). Analysis is done to measure statistical significance of the trend of resistance to fluoroquinolones (Table 9).

Table 9: Factors associated with fluoroquinolone resistance in ICL 2013-2016.

Variable		Resistance Count (%)	Significance level (2-sided)	Crude OR	Adjusted OR	95% Confidence Interval (CI)	
						Lower	Upper
Sex	Male	1402 (47.0)	0.25	1.13	0.94	0.86	1.04
	Female (*Reference*)	2163 (44.1)					
Species	Gram Negative	2817 (46.1)	0.85	1.18	0.99	0.86	1.04
	Gram Positive (*Reference*)	1031 (42.0)					
Age	Less than 15 years (*Reference*)	212 (22.8)	0.00				
	15-29 years	542 (35.1)	0.00	1.83	1.80	1.49	2.17
	30-44 years	697 (38.5)	0.00	2.12	2.07	1.72	2.48
	45-59 years	816 (53.4)	0.00	3.87	3.86	3.21	4.64
	60 years and above	1298 (62.4)	0.00	5.61	5.63	4.71	6.73
Year	2013 (Reference)	791(40.4)	0.00				
	2014	886 (45.1)	0.01	1.21	1.20	1.05	1.38
	2015	926 (49.0)	0.00	1.42	1.4	1.2	1.6
	2016	962 (46.3)	0.11	1.27	1.12	0.98	1.28
	Constant		0.00		0.26		

6.6. Qualitative Findings contributing factors to fluoroquinolone

This section presents summary of qualitative findings. In-depth interview was employed to generate information on the perspective of pharmacists and physicians. The principal investigator conducted the in-depth interview which lasts an average of 40 minutes ranging from 40-60 minutes. The interview was conducted in Amharic with the aid of recorder. Also the principal investigator took note during interview on emerging new themes. In cases of ambiguity, the principal investigator clarifies the issues raised instantaneously. The interviews were categorized under pre- identified 4 themes. The four themes were dispensing and prescribing practice, trend of bacterial resistance, contributing factor for antibacterial resistance, and solutions to contain antibacterial resistance.

A total of 20 professionals participated in the in-depth interviews. Nine interviewees were female and the remaining 11 were male. Regarding place of work, 13 interviewees were from public health facilities and 7 were from private health facilities. A total of 11 health facilities were covered; a health center and 3 hospitals from government institutions; 2 pharmacies, 2 drug

stores and 2 clinics and one laboratory from private health facilities. The average age of participants was 32 years with a minimum of 24 years and a maximum of 45 years. Key informants included 6 pharmacists; 4 druggist, 2 nurses (BSc), 8 physicians (6 GPs and 2 specialists). Majority had work experience of 5 to 9 years.

Dispensing and prescribing practices

Most (n=7) of the physician key informants informed that prescribers prefer broad spectrum antibacterial and second line drug treatment. That means prescriptions might not comply with the standard treatment guideline for Ethiopia or essential drug list of the health facilities. The physicians said they tend to prescribe broad spectrum antibiotics due to the fact that there were either no complete diagnostic services or frequent stock out of drugs. This was emphasized by one of the participants:

"…… Our main purpose here is to save lives of patients. To do so, we have to use the available resource for better clinical decision making. To me broad spectrum antibiotics are usually first choice, increasing confidence on patient clinical outcome tested by experience of the past several years (AMR 013)."

In addition to the above fact, physicians tend to treat diseases without following standard treatment protocol. High work burden of physicians due to high physician to patient ratio was one of the factors to order broad spectrum. One of the participants underscored that:

"…… we usually prescribe drugs that were believed to cure a certain disease from past experience. These days the number of patients coming to our hospital increased dramatically. As the number patients to be served at once increases, I prefer to treat some patients from my past experiences than to be at risk of error …… (AMR 014)"

Most (n=13) participants agreed that ownership of the health facility also influences prescribing and dispensing practices. Government owned dispensing facilities were dispensing drugs to patients coming with prescription; they were less costly and tend to follow rules and regulations. However, participants having private dispensing experience said that they focus on business and provide drugs without prescription. Private pharmacies were better than government dispensing facilities on providing counseling service. This is substantiated by the participant that:

"I am working for both the public and the private health facilities. I witness that the behavior of private clinics and pharmacies was driven on business basis rather than the treatment protocol, while the behavior of government health facilities including mine were driven by number of patients. If the number of patients attending government hospital is high; the level of care in the dispensaries will be minimized. In a private pharmacy if a patient requested ciprofloxacin (with a brand name) without a prescription, I will sell the drug not to lose the patient otherwise (AMR 003)."

Trends of antibacterial resistance

The findings demonstrate that bacterial resistance to antibiotics was a common phenomenon. The resistance was common to antibiotics such as amoxicillin, cotrimoxazole, tetracycline and ciprofloxacin. Majority (n=14) of participants mentioned typhoid, urinary tract infection, and upper respiratory tract infections as diseases with common occurrence of antibacterial resistance. This was demonstrated by one of the participants saying:

"*I encountered a patient with UTI infection visited five times with worsening prognosis. We suspected antibacterial resistance and send the patient for sensitivity test. The patient was found to be resistant for almost all antibiotics including Fluoroquinolones. It was very difficult to treat the patient and took a lesson to consider antibacterial resistance in our day to day clinical duties (AMR 012)."*

Slightly less than half of the participants emphasized the challenge of antibacterial resistance in their daily practices. The challenges identified from participants were poor prognosis of patients' health, unable to treat patients with the available first line treatment, complicated management of patients, and increased burden on hospital service due to longer average length of stay and repeated visits. This was further substantiated by one of participants:

"….. These days' patients were complaining and getting into conflict with our staff due to the nonresponse to the dispensed drugs. They insist that we should change the drug into other effective ones. Some patients even did not trust our service (AMR 002)."

The finding demonstrates that there was an increasing trend of antibiotic resistance in general and fluoroquinolones resistance in particular. Majority (n=13) participants agreed on an

increasing resistance pattern over years. Participants underscored that patients with older age, female and low income were more likely to acquire antibacterial resistance than other segment of the population. This was supported by one participant from private sector saying that:

"I have encountered many women patients requesting for antibacterial drug for UTI without prescription. My recent experience was that a woman came to our facility to get norfloxacin. She said that whenever her UTI pain starts, I take this drug. She just took the drug before 15 days of visiting our facility (AMR 007)."

On contrary to this the rest of the KIs argued that patients of younger age, male and with high socioeconomic status were likely to acquire antibacterial resistance. The participant elaborated that male patients ignore their disease and remain asymptomatic which leads to drug resistance. Similarly participants said patients with high socioeconomic status were taking concomitant drug and did not adhere to full course treatment. They tend to stop taking the antibiotics as the symptoms get relieved. They were also likely more educated and prefer the drug by their choice. Young patients that had repeated occurrence of disease such as diarrhea may acquire drug resistance. This was supported by participant that:

".....I have seen drug resistance in many young patients. It is very difficult to think of drugs to prescribe in the future as there is an increasing trend of drug resistance…… (AMR 001)"

Contributing factors for antibacterial resistance

The qualitative findings revealed that physicians, pharmacists and patients' behaviors contributed to fluoroquinolone resistance. Over the counter (OTC) sells of Fluoroquinolones was underscored by all participants as a major contributing factor for antimicrobial resistance in general and fluoroquinolones resistance in particular. Moreover, the participants from private dispensary outlets said the pharmacists usually dispense partial dose of drugs (if the patients requested) insisted of the full dose prescribed by physicians. In relation to this one of the KIs stated that:

"I am sure that all pharmacy professionals clearly know the implication of selling antibiotics without prescription. You know the competition is becoming very tough; we need to survive, so we need to fulfill patients demand, otherwise they will satisfy their OTC need from the next

pharmacy. However, I try to convince patients before selling partial dose of drugs to satisfy our business need as well as their health need. If they insist to get portion of the drug due to economic reason, I usually advise them to complete their treatment (AMR 009)."

The behaviors of prescribers was also identified by majority (n=12) of participants as one of the key factors contributing to antibacterial resistance. The participants said that physicians relaying on empirical treatment and not using updated information as the reason for antibacterial resistance. However, there were few KIs who argued to the contrary that empirical treatment is less common and physicians take appropriate considerations before such clinical decisions reached. A KI said the following in support of this argument:

"I strongly believe that physicians' empirical treatments' contribution to antimicrobials resistance is insignificant as compared the contribution of the widespread OTC dispensing practices. You should give priority to the problem of OTC dispensing, dispensing of only one to two days dose of drug insisted of full course treatment and dispensing of drug without proper counseling. ……. What irritates me is that physicians are blamed for others problem. (AMR 015)"

Majority of the KIs agreed that misuse of antibiotics by the public was one of the major reasons for Fluoroquinolone resistance. They said that the community discuss at home and take antibiotics by themselves. Usually patients stop taking medicine when they feel the symptom of the disease is relieved. Most of the time patients stop taking drugs on day 4 or 5 that were prescribed for 7 days. Moreover, patients do not want to visit clinics for the disease symptoms treated effectively in the past and did not want to waste their energy or money for the costly health care. This is in alignment with a participant:

"Patients requesting for antibiotics without prescription usually say. Brother, your job is to avail the drug ….. I know my drug; my doctor always prescribes this one for my sickness. Why do you bother me spending my time and cost…. (AMR 006)"

Bacterial resistance containment

Participants identified that strengthening the regulation, creating awareness to the community, updating of health professionals on resistance, and establishing microbiology laboratory at least

at hospital level should be implemented to curb the increasing trend of antibacterial in general and Fluoroquinolone resistance in particular.

All participants agreed regulatory enforcement as solution to contain antibacterial resistance. They said regulatory system was characterized by weak low enforcement. The priority law enforcement areas were controlling of antibacterial OTC treatment and regular clinical audits to enforce compliance with standard treatment guideline or clinical standard.

The findings revealed that community self-medication practice can only be improved with awareness creation and social mobilization movement. Majority (n=15) of participants illuminated cultural practice, the increasing price of health care and peer pressure as contributors to self-medication. This is underscored by the participant that:

"As you see, the public health facilities do not provide complete service delivery for patients. Either there is interruption of service at laboratory, pharmacy or imaging services. This leads to costly service at private facilities. Patients particularly with low socio economic status prefer self – medication instead of following the right path of getting service (AMR 014)"

The participants said that there is no standard laboratory service to perform antibacterial sensitivity tests in the majority of the health facilities. In addition, there is no updating of information related to bacterial susceptibility to make evidence based clinical decisions.

7. Discussion

In this retrospective record review the common bacteria isolates identified and tested against fluoroquinolones' resistance were *E.coli, Klebsiella, Staphylococcus, Pseudomonas, Enterobacter, and Enterococcus*. Most of the identified bacteria's were gram negative; of which the majority of isolates were from the genus *Enterobacteriaceae*. *Enterobacteriaceae* causing community acquired or healthcare associated urinary tract infections and intra-abdominal infections, exceeding 50% in some parts of the world, particularly in Asia (Dalhoff, 2012)**.** Ko *et al.* (2009) and Lu *et al.* (2012) also showed fluoroquinolones resistance identified for gram negative bacteria. This is because fluoroquinolones were most of the time indicated for diseases caused by gram negative bacteria.

The present study indicated that most of the major isolates were uropathogens (from urine), accounting 79.1%. This might be due to the fact that more cases in the study were females (62.2%) which are more vulnerable for urinary tract infections. This is consistent with the findings of other previous researches (Schito *et al*., 2009; Suk *et al*., 2014). For example, a study by Schito *et al*. (2009) showed that E. *coli,* other *Enterobacteriaceae,* and *Enterococcus* were the primary etiology of uncomplicated UTIs, with *E. coli* accounting more than 75% of the isolates. Foxman, 2002 as well as Russo and Johnson (2003) also stated that E. coli is the most common agent of urinary tract infections (UTIs) in all parts of the world.

The overall bacterial resistance to fluoroquinolones in the present study was 45.2%. This finding was higher compared to the other study done in North America (Babinchak *et al*., 2013). In this study bacterial resistance varies by type of fluoroquinolones. Our study revealed that nalidixic acid(63.3%) developed high resistance followed by norfloxacin (44.4%) and ciprofloxacin (42.8%). A study by Ohieku *et al*., (2013), Somashekara *et al.*, 2014 and Akya *et al*., 2015 showed similar result for fluoroquinolones resistance. Consequently, fluoroquinolones might not be the choice of treatment which leads to prescribing of other second line drugs which are costly.

The qualitative part of our findings indicated that the major reasons for fluoroquinolones resistance were OTC sells of antibacterial the nature of the drug (broad spectrum and frequency of dosing), empirical treatment, inaccessibility of culture and sensitivity test and irrational self-medication practices. This finding was also supported by other studies done in Ethiopia (Gebretekle *et al*., 2016). Interviewed physicians, point out that private pharmacies were

dispensing fluoroquinolones upon the request of patients without prescriptions from physicians. Similarly the World Health Organization indicated that over the counter use of drug as one of the major contributing factor for drug resistance (WHO, 2001). The present study identified also that high level of fluoroquinolones resistance is mainly caused by irrational prescribing and dispensing practices and calls for the need for advocacy and promotion regarding fluoroquinolones resistance. The study also suggests improving accessibility of culture for bacterial susceptibility test as well as strong regulatory measures need to be taken to reduce OTC availability of antibacterial in retail outlets.

This study demonstrated that isolates of *Enterococcus* and *E.coli* showed higher resistance to fluoroquinolones. Nearly half of the *E.coli* isolates were resistant to ciprofloxacin and norfloxacin. Similarly a study conducted in Hawassa by Gizachew *et al.* (2013) showed that the resistance of *E.coli* to ciprofloxacin was 43.8%. A higher extent of resistance by *E.coli* to Norfloxacin was observed in a tertiary care hospital in north India (Niranjan, 2014). Sanchez *et al.,* 2012 also revealed that 20–50% of E. coli isolates are now resistant to the first-line of antibiotics. Several studies revealed that fluoroquinolone resistant *E. coli* has been reported increasingly during the last decade in both the hospital environment and the community, which may ultimately limit the utility of these broad-spectrum agents Consequently, the choice of empiric treatments for UTIs has now become challenging (Chaniotaki *et al*., 2004; Garau *et al.,* 1999; Van *et al*., 2001 Sharma *et al*., 2016). Fluoroquinolone resistant *E.coli* strains often also show resistance to other antibiotics (Chaniotaki *et al*., 2004; Garau *et al*., 1999; Van *et al*., 2001).

The cause for *E.coli* resistance could be over use of fluoroquinolones (Suk *et al.*; 2014). Multi drug resistant (MDR) strains of *Escherichia coli*, particularly to fluoroquinolones, are the major causative agents for hospital acquired infections, as well as epidemics linked to gastrointestinal and urinary tracts in the non-hygienic communities of most developing countries (Rath *et al.*, 2015). Therefore, it is important to identify the susceptibility of isolates when treating hospital acquired infection using fluoroquinolones. *Pseudomonas* and *staphylococcus* showed high resistance to nalidixic acid. This is expected because both isolates develop clinically important level of resistance by single mutation (Hooper, 2001). This implies that fluoroquinolones should be cautiously used in treating infections caused by *Pseudomonas* and *staphylococcus species.*

Hence, different factors including prior patients' fluoroquinolone use history need to be considered before prescribing fluoroquinolones.

In general, fluoroquinolone resistance increases as age of patient's increases, it nearly triples between the ages less than or equal to 15 years compared to age category greater than 60 years (22% vs. 62%). This finding concurs with other studies which identified age as a risk factor for fluoroquinolone resistance (Campa, 2004; Suk *et al.*, 2014 and Fasugba *et al*., 2016). The reasons for the dramatic increment of resistance with age might be prior exposure to fluoroquinolones; recurrent UTI; the presence of complicated urinary tract infection, decreased immunity and etc. More than one in five bacterial isolates were resistance for age group less than 5 years. The reason of resistance on children might be due to the transmission of mutant bacteria from their mother, contaminated environment or the transmission of the resistant bacteria from animal product such as egg (Johnson *et al.*, 2007).

The present study also indicated that Fluoroquinolones resistance was higher among males than females. Similar studies found out that among male patients, resistance rates to most antimicrobials were high (Zec *et al.*, 2016 and Boyd *et al*., 2008). According to key informant physicians, males stay for longer time without being symptomatic for UTI infection and come to clinic at later stages. This study revealed that resistance increased with age. Several studies showed high resistance rates were observed for all antimicrobials among older patients (Campa, 2004, Boyd *et al*., 2008, Zec *et al.*, 2016, Gururaju *et al.*, 2016 and Mandal *et al.*, 2012). This resistance might be due to pre exposure of fluoroquinolones as well as acquiring resistant bacteria from environment.

It is alarming that in the past four years, there was a general increasing trend of fluoroquinolones resistance by a mean percentage points of nearly 5. Several studies also demonstrated that fluoroquinolone resistance rates have continued to increase, affecting patient management (Jun et al., 2013 Rodrigues *et al*., 2016). An increasing resistance to broad-spectrum antibiotics such as fluoroquinolones was due to an overconsumption of these groups and the parallel development of cross resistance to other antibiotics (Cassier, 2011 and Sharma *et al*.,2016) and necessitating the need for changes in the current treatment guidelines (Keddy, 2010,; Kuntaman *et al*., 2005).

Given the increased resistance to these drugs, urine culture and antimicrobial susceptibility testing are essential for the treatment of UTIs.

This finding suggests revision of treatment guideline for urinary tract infections especially in treatment of older people in central Ethiopia. In addition to this, the innovation of new antibiotics has been declining in the world suggesting that prudent use of available antibiotics is the only option to delay the development of resistance (Komp *et al*., 2003).

8. Limitations

It was originally planned to analyze the data from 2012 to 2016 but due to lack of document for the year 2012 the analysis used only four years period. In this retrospective study, there is no consideration of patient's demographic data like socioeconomic status etc., and clinical symptoms, complicated versus uncomplicated UTI, which are surely the limitations of this study. The study also did not address patients' perception and use of fluoroquinolones, which could have given more information with respect to factors contributing to resistance. Most of the samples are from Addis Ababa therefore the result may not be representative for other regions.

9. Conclusion

There was relatively high bacterial resistance fluoroquinolone demonstrated by the most predominant isolates, *E.coli, Enterobacter, Enterococci, Klebsiella, Pseudomonas* and *Staphylococcus*. There is high resistance in people with age of sixty years and above. The trend of resistance for fluoroquinolone increases over the three years except 2016. Misuse and overuse of fluoroquinolone by prescribers, dispensing of fluoroquinolones over the counter by pharmacy professionals and use of fluoroquinolone for self-medication by patients were raised as a contributing factor for the fluoroquinolone resistance. Resistance against fluoroquinolones was increasing therefore further study need to be conducted to identify determinants. Giving awareness for the public, strengthening regulation on antibiotic use, giving refreshment training for the prescribers and dispensers and establishing microbiology service at least in hospitals are among the priority containment solutions that should be implemented to decrease the spread of fluoroquinolone resistance.

10. Recommendations

The Ministry of Health has to conduct advocacy sessions, and coordinate further research on factors that were not addressed by this study that will lead to increase the resistance against fluoroquinolones.

Update physicians on susceptibility pattern of bacteria by improving dissemination of an up-to-date antibiogram for prescribers, and rational prescription to improve use of fluoroquinolones.

Prescribers should prescribe fluoroquinolones in caution for older people. Given the increased resistance to these drugs, urine culture and antimicrobial susceptibility testing are essential for the treatment of UTIs in this group.

Promoting ethical practice would also help rationalizing the prescribing and dispensing practice by professionals

Strengthen a mechanism to control OTC sale of fluoroquinolones and promote rational dispensing.

Continuous awareness raising and behavioral change communication has to be devised towards rational use to the community.

Improving accessibility of culture for bacterial susceptibility test

11. References

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12. Annexes

Annex 1

Data abstraction format for Retrospective survey on fluoroquinolones resistance pattern and trend in ICL, Ethiopia

S.no	Year	sex	Age	Address	Specimen	Isolates	Nalidixic acid	Ciprofloxacin	Norfloxacin

Response

S. Sensitive

I. Intermediate

R. Resistance

Data collector name and signature_____

Annex 2

Interview guide for key informants

Greeting I am _____ and I am here to obtain information for the study for the fulfillment of MSc in pharmacoepidemiology and social pharmacy program from Addis Ababa University. After hearing the following general things about the study, if you are willing we will precede the interview which lasts only 30 minutes.

Title of the study: Assessment of Bacterial resistance trends and contributing factors to Fluoroquinolone in Ethiopia.

Benefit: This study will not give direct benefit to the participants but the information which will be gained from the participant will help federal ministry of health and other stakeholders.

Risk: The study will not impose any risk on participant except spending few minutes for the interview.

Right of the respondents: Participation in the study is voluntary. At any time you can quite from giving answer for the questionnaire that you are not willing to answer or even you can stop.

Confidentiality: The information you give will not be accessed to anybody other than the investigator and any information that you will give kept confidential. Also your name and personnel identifier will not be indicated.

You can ask any thing for clarity. Are you willing to participate in the study?

Name of data collector _____Signature _____

Thank you for your time and energy

Interview guide for prescriber

Part 1: Socio-demographic Characteristics

No	Question /variable	Response
1	Date of interview (dd, mm, yyyy)	
2	Code of the interview	
3	Age in year	
4	Gender	1. Male 2. Female
5	Highest education level completed	1. B.Sc. 2. M.D 3. Specialist 4. Sub-specialists 5. Other
6	Years of professional practice	

Part 2: Qualitative question

1. How do you describe the overall antibacterial prescribing practice in your organization? And also outside your organization?

   Probe: What do you think about the trend of prescribing anti-bacteria's? Compliance to STG or drug list specific to this hospital, use of broad spectrum antibiotics over time, use anti-bio gram to prescribe antibiotics… is there empirical treatment? If yes, what do you think is the rationale behind empirical treatment?
2. How do you describe the prescribing practices specific to fluoroquinolones in your organization? What about other than your organization?

   Probe: for what type of diseases are you using fluoroquinolones as first line drugs? What do you think about the trend of prescribing fluoroquinolones? Compliance to STG or drug list specific to this hospital, use of broad spectrum antibiotics over time…is there empirical treatment? If yes, what do you think is the rationale behind empirical treatment?
3. What is your opinion on the resistance of bacteria's to common antibiotics?

Probe: Do you recognize bacteria's resistance to common antibiotics in your area of practice? If yes, to what types of antibiotics do you think resistance is most common? Do you face any challenge in your daily practice as a result of antibacterial resistance? If yes what was the challenge?, how do you describe its resistance pattern over years? Do you think it a major problem to your institution and/or the country at large?

4. What do you think about resistance of bacterial to fluoroquinolones?

   Probe: Did you diagnose patients with fluoroquinolones resistance in your practice? Do you face any challenge in your daily practice as a result of fluoroquinolones resistance? If yes what was the challenge?

5. What do you think is the contributing factors to antibacterial resistance in general?

Probe: Poor infection control, empirical treatment, misuse of antibiotics by physicians, misuse of antibiotics by the public, poor quality of antibiotics, expired antibiotics, counterfeit and adulterated drugs, etc…

6. What do you think is the contributing factors for fluoroquinolones resistance in particular?

Probe: broad spectrum, social life style and patient behavior, safety, effectiveness, availability, cost, less side effect….

7. What possible measures do you recommend for better antibacterial resistance containment practices?

   Probe : Infection prevention and control, rational prescribing ,rational use, updating oneself and other health professional about antibacterial resistant updates and containment mechanism, teaching patients and public about antibacterial resistant updates and containment mechanism, conducting research on the area

8. What do you suggest solution to reduce resistance to fluoroquinolones specifically?

   Probe: The promoting factors and limiting factors for fluoroquinolones resistance containment? If there is anything you would like to add

Interview guide for Dispenser

Part 1: Socio-demographic Characteristics

No	Question /variable	Response
1	Date of interview (dd, mm, yyyy)	
2	Code of the interview	
3	Age in year	
4	Gender	1. Male 2. Female
5	Highest education level completed	1. Diploma 2. B, pharm 3. M. Sc in pharmacy 4. Other
6	Years of professional practice	

Part 2: Qualitative question

1. How do you describe the overall antibacterial dispensing practice in your organization? And also outside your organization?

   Probe: What do you think about the trend of dispensing of anti-bacteria's? Compliance to STG, a medicines formulary or drug list specific to this hospital use of broad spectrum antibiotics over time, use of generic vs. brand medicine, availability of 1st line drug or drug substitution, patient preference for specific drug or dosage form.
2. How do you describe dispensing practice specific to fluoroquinolones in your organization? What about other than your organization?

   Probe: for what type of diseases are you using fluoroquinolones as first line drugs? What do you think about the trend of dispensing of fluoroquinolones? Compliance to STG or drug list specific to this hospital, use of broad spectrum antibiotics over time…is there empirical treatment? If yes, what do you think is the rationale behind empirical treatment?
3. What is your opinion on the resistance of bacteria's to common antibiotics?

Probe: Do you recognize bacteria's resistance to common antibiotics in your area of practice? If yes, to what types of antibiotics do you think resistance is most common? Do you face any challenge in your daily practice as a result of antibacterial resistance? If yes what was the challenge? How do you describe its resistance pattern over years? Do you think it a major problem to your institution and/or the country at large?

4. What do you think about the extent of resistance of bacterial to fluoroquinolones?

   Probe: Do you recognize bacteria's resistance to common fluoroquinolones in your area of practice? If yes, to what types of fluoroquinolones do you think resistance is most common? How much did you encounter in the last year, what type of diseases, what type of patient characteristics (age, sex, education, residence, socio-economic status…..)

5. What do you think is the contributing factors to antibacterial resistance in general?

Probe: Empirical treatment, Misuse of antibiotics by physicians, Misuse of antibiotics by the public, Poor quality of antibiotics, Expired antibiotics, Counterfeit and adulterated drugs, etc…

6. What do you think is the contributing factors for fluoroquinolones resistance in particular?

Probe: broad spectrum, social life style and patient behavior, safety, effectiveness, availability, cost, less side effect, if other, please mention….

7. What possible measures do you recommend for better antibacterial resistance containment practices?

   Probe : Infection prevention and control, rational dispensing ,rational use, updating oneself and other health professional about antibacterial resistant updates and containment mechanism, teaching patients and public about antibacterial resistant and containment mechanism, conducting research on the area

8. What do you suggest solution to reduce resistance to fluoroquinolones specifically?

   Probe: The promoting factors and limiting factors for fluoroquinolones resistance containment? If there is anything you would like to add