Original Article

A Comparative Study on Susceptibility of Enterobacteriaceae to Six Quinolones in Yaounde

Étude Comparative de la Sensibilité des Entérobactéries à Six Quinolones à Yaoundé

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ABSTRACT

Introduction. Enterobactericeae are associated with many types of infections including abscesses, pneumonia, meningitis, septicaemia, and intestinal, urinary and wound infections. Fluoroquinolones (FQs) represent the drug of choice for the treatment of a wide range of human infectious diseases caused by enterobacteriaceae. This study aimed at comparing the susceptibility of six quinolones (Qs) of different generations that are often used in the empirical treatment of cases of suspected enterobacterial infections where susceptibility testing is not always systematic.

Methodology. Three hundred enterobacteriaceae species were isolated from 13 different clinical specimens. Identification was done using Api 20E. Susceptibility testing was performed using the Kirby Bauer Disc Diffusion method using two Qs of first generation; nalidixic acid and pipemidic acid, and four FQs; two second generation; norfloxacin and ciprofloxacin, one third generation; sparmofloxacin and one fourth generation; moxifloxacin.

Results. The resistance of the different species to the first generation Qs was: *Klepsiella* 38/99(38.4%); *Escherichia* 38/108(35.2%); *Enterobacter* 7/24(29.8%); *Proteus* 5/24(20.8%); *Serratia* 6/21(28.6%); *Salmonella* 1/9(11.1%); *Citrobacter* 1/8(12.5%). The resistance to the FQs was: *Klepsiella* 31/99(31.3%); *Escherichia* 30/108(27.8%); *Enterobacter* 4/24(16.7%); *Proteus* 2/24(8.3%); *Serratia* 6/21(28.6%); *Salmonella* 0% and *Citrobacter* 1/24(12.5%); Overall, 99/300(33.0%) of isolates were resistant to the Qs and 77/300(25.7) to the FQs (P-value 0.05).

Conclusion. This study has shown that overall there is no significant difference in the susceptibility between the Qs of the first generation and the FQs in the treatment of enterobacterial infections. The high of percentage quinolone resistance makes it necessary for us to use a rational in prescribing these drugs.

Key Words: Quinolones, Fluoroquinolone, Resistance, Enterobacteriaceae

RÉSUMÉ

Introduction. Les Entérobactéries sont associées à plusieurs types d'infections incluant les abcès, les pneumonies, les méningites, les septicémies et les infections intestinales, urinaires et des plaies. Les Fluoroquinolones (FQs) représentent l'antibiotique de choix dans le traitement d'une large gamme de maladies infectieuses humaines causées par les entérobactéries. Cette étude avait pour but de comparer la sensibilité de six quinolones (Qs) de différentes générations qui sont très souvent utilisées dans le traitement empirique des cas suspects d'infections entérobactériennes où l'étude de la sensibilité aux antibiotiques n'est pas toujours systématique.

Méthodologie. Trois cent espèces d'entérobactéries ont été isolées à partir de 13 types d'échantillons cliniques. L'identification a été faite en utilisant Api 20E(Biomérieux). La sensibilité aux antibiotiques a été faite en utilisant la méthode de diffusion des disques de Kirby Bauer. Nous avons utilisé deux Qs de première génération : acide nalidixique et acide pipémidique, et quatre FQs : deux de deuxième génération : norfloxacine et ciprofloxacine, un de troisième génération : la sparmofloxacine et une de quatrième génération : la moxifloxacine.

Résultats. La résistance des différentes espèces aux Qs de première génération était : *Klebsiella* 38/99(38.4%); *Escherichia* 38/108(35.2%); *Enterobacter* 7/24(29.8%); *Proteus* 5/24(20.8%); *Serratia* 6/21(28.6%); *Salmonella* 1/9(11.1%); *Citrobacter* 1/8(12.5%). La résistance aux FQs était: *Klebsiella* 31/99(31.3%); *Escherichia* 30/108(27.8%); *Enterobacter* 4/24(16.7%); *Proteus* 2/24(8.3%); *Serratia* 6/21(28.6%); *Salmonella* 0% et *Citrobacter* 1/24(12.5%); En tout, 99/300(33.0%) des isolats étaient résistants aux Qs et 77/300(25.7) aux FQs (P-value 0.05).

Conclusion. Cette étude a montré que dans l'ensemble, il n'y a pas de différence significative de sensibilité entre les Qs de première génération et les FQs dans le traitement des infections entérobactériennes. Le taux élevé des résistances des quinolones rend nécessaire la prescription rationnelle de ces médicaments.

Mots clés: Quinolones, Fluoroquinolones, Resistance, Entérobactéries

INTRODUCTION

Enterobacteriaceae may account for approximately 80% of clinically significant isolates of gram-negative bacilli and they may also account for 50% of all clinically significant isolates in clinical microbiology laboratories. They are associated with many types of infections including abscesses, pneumonia, meningitis, septicaemia, and intestinal, urinary and wound infections [1, 2].

Fluoroquinolones (FQ) represent the drug of choice for the treatment of a wide range of human infectious diseases caused by Enterobacteriaceae. Their use now accounts for about 11% of overall prescriptions of antimicrobials in human medicine and one of them, ciprofloxacin, is the most used antibiotic in the world [3,4]. FQ were a major therapeutic advancement in the 1980s because they have 100-fold greater activity than their parent compound, nalidixic acid [5].

The first quinolone, nalidixic acid was introduced in 1962. Since then, structural modifications have resulted in second-, third-, and fourth-generation fluoroquinolones (FQ), which have improved coverage from only gram negative to now gram-positive organisms (Oliphant et al., 2002). The main chemical feature that distinguishes fluoroquinolones from quinolones is the presence of fluorine at position six of the carboxylic acid moiety [6, 7, 8].

Early predictions suggested that emergence of FQ resistance, particularly among the Enterobacteriaceae, was very unlikely. Subsequent reports noting the emergence of FQ resistance in the Enterobacteriaceae were of great concern, given that these pathogens cause a substantial proportion of serious hospital-acquired infections [9]. Early researchers thought that FQ resistance was unlikely to evolve, largely because resistant *Escherichia coli* mutants are exceptionally difficult to select in vitro and because plasmid-mediated quinolone resistance remained unknown even after 30 years of nalidixic acid usage [5].

Today antimicrobial resistance is one of the major problems confronting clinicians in their work. The increasing resistance to most antimicrobials complicates the use of antimicrobial agents and the control of infectious diseases [4, 10]. The emergence of strains showing resistance to several quinolone antimicrobial agents is a public health concern [11].

Although many studies exist identifying resistance in enterobacteriaceae to quinolones and FQ, few comparative data exists. To address this we performed a cross-sectional study comparing the reaction of enterobacteriaceae to 2 Qs and 4 FQs of different generations that are often used in the empirical treatment of cases of suspected enterobacterial infections where susceptibility testing is not systematic.

METHODS

This was a cross-sectional descriptive study of three hundred enterobacteriaceae isolates. One isolate was collected per patient from one of 13 potential clinical specimens: Urine, vaginal/cervical swap, hemoculture, urinary catheter, pus, stool, urethra swap, sputum, bed sore, bone fragment, pleural fluid, seminal fluid and wound.

These specimens were innoculated on Eosin Methylene Blue (EMB) Agar, incubated for 18-24 hours at 37°C. Colonies were Gram-stained using standard laboratory procedures. Gram-negative bacilli were then identified using the API 20E identification kit (BioMérieux SA, Lyon, France).

Antibiotic susceptibility was done by the Standardized Kirby-Bauer disc diffusion method on Mueller-Hinton agar for two quinolones; nalidixic acid (NA) and pipemidic acid (PI), and four fluoroquinolones; norfloxacin (NOR), ciprofloxacin (CIP), sparmofloxacin (SPX) and moxifloxacin (MXF). The quality control of discs used was performed using the following reference strain; *E. coli* ATCC 25922. Phenotypic disc determination was done using the Clinical and Laboratory Standard Institute [12] (CLSI) (CLSI, 2007) performance standards for antimicrobial susceptibility testing.

All data generated was entered into an excel spread sheet and analysed using Epi Info Version 3.2 of February 2004. Proportions were compared using Chi-Square tests or Fisher's exact tests, as appropriate. The level of statistical significance was set at a p-value ≤ 0.05.

Authorisation to carry out this study was obtained from the ethical committee of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I.

RESULTS

Out of the 300 enterobacteriaceae isolates identified, the distribution of the species by genera was; *Escherichia* 36%, *Klebsiella* 33%, *Enterobacter* 8%, *proteus* 8% *Serratia* 7%, *Salmonella* 3%, *Citrobacter* 2.7% and others 2.3%.

Resistance of the isolates to all Quinolones/Fluoroquinolones (Q/FQ) was 25.7% (77/300). The frequency of resistant isolates to the various Q/FQ was as follows: nalidixic acid 104/300(34.7%), pipemidic acid 114/300(38%), norfloxacin 86/300(28.7%), ciprofloxacin 84/300(28%), sparfloxacin 92/300(30.7%) and moxifloxacin 92/300 (30.7%).

The resistance with respect to genera was; *Klebsiella* 31/99(31.3%); *Escherichia* 27/108 (25%); *Enterobacter* 7/24(25%); *Serratia* 6/21 (28.6%); *Proteus* 2/24(8.3%), Salmonella 0/9(0%), Citrobacter 1/8(12.5%) others 3/7 (42.9%).

There were 77/300(25.7%) isolates resistant to all the six Q/FQ used; 4/300(1.3%) were resistant to five and four; 3/300(1%) were resistant to three, then 13/300(4.3%) were resistant to two and 12/300(4%) were resistant to only one Q/FQ. Meanwhile, 187/300(62.3%) were sensitive to all six Q/FQ.

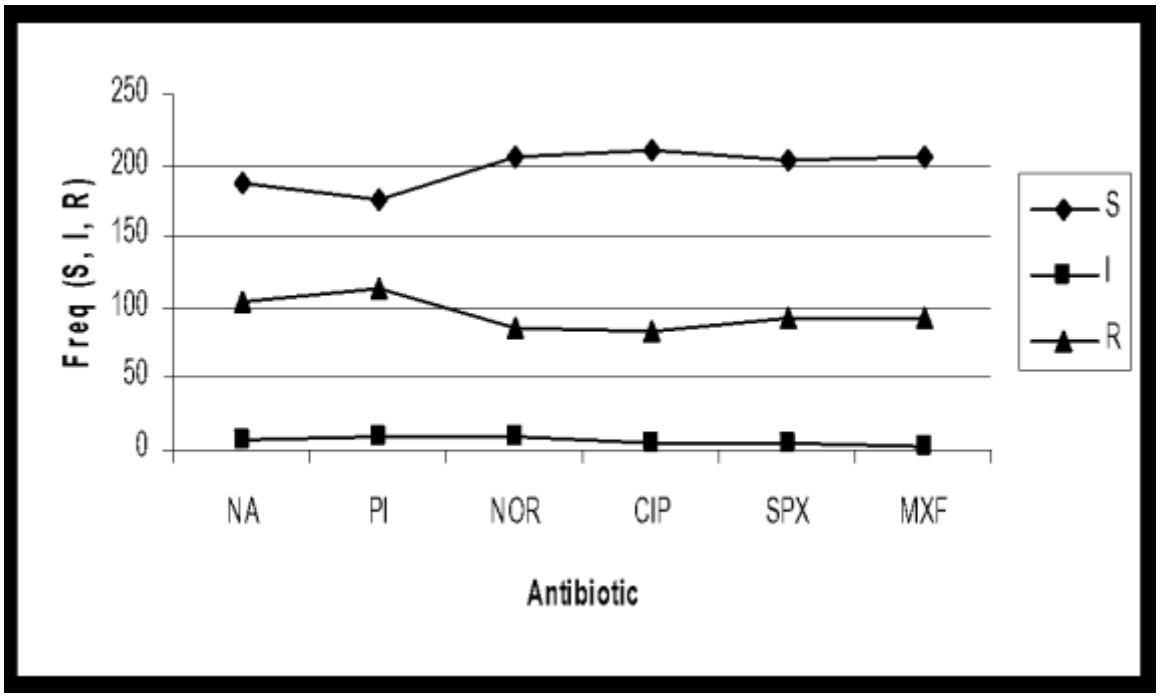
The resistance of the different species to the first generation quinolones (Q), nalidixic acid and pipemidic acid, was: *Klepsiella* 38/99(38.4%); *Echerichia* 38/108(35.2%); *Enterobacter* 7/24(29.8%); *Proteus* 5/24(20.8%); *Serratia* 6/21(28.6%); *Salmonella* 1/11(11.1%); *Citrobacter* 1/12(12.5%). The resistance to the fluoroquinolones (FQ) that is ciprofloxacin, norfloxacin, sparmofloxacin and moxifloxacin was: *Klepsiella* 31/99(31.3%); *Echerichia* 30/108(27.8%); *Enterobacter* 4/24(16.7%); *Proteus* 2/24(8.3%); *Serratia* 6/21(28.6%); *Salmonella* 0/9(0%) and *Citrobacter* 1/8(12.5%); Overall, 99/300(33.0%) of isolates were resistant to the Qs and 77/300(25.7) to the FQs (P-value 0.05). These results are depicted in table 1

Table 1: The comparison of the resistance of enterobacteriaceae species to the quinolone (Q) and Fluoroquinolone (FQ)

	Q n(%)	FQ n(%)	P-VALUE
Escherichia Spp (n=108)	38 (35.2	30 (27.8	0.24
Klebsiella Spp (n=99)	38 (38.4	31(31.3	0.29
Enterobacter Spp (n=24)	7(29.2	4 (16.7	0.30
Proteus spp (n= 24)	5 (20.8	2(8.3	0.22
Serratia spp (n=21)	6(28.6	6(28.6	1.00
Salmonella spp (n=9)	1(11.1	0 (0	0.30
Citrobacter spp (n=8)	1(12.5	1(12.5)	1.00
Others (n=7)	3(42.9	3(42.9	1.00
Total (n=300)	**99(33.0**	**77(25.7**	**0.05**

The susceptibility (Frequency of Sensitive, intermediate and Resistance) variation of the Qs and FQs shows that the resistance peaks at pipemidic acid and is lowest at norfloxacin as seen in figure 1.

The cross resistance between sparmofloxacin and moxifloxacin was 100%; nalidixic acid and pipemidic acid 98.1%; moxifloxacin and Nalidixic acid 94.57%. These results are summarised on table 3



S=Sensitive; I=Intermediate; R=Resistance

Figure 1: Variation of the susceptibility with respect to the generation of quinolones and fluoroquinolones

Dates of first production

1st generation	2nd generation	3rd generation	4th generation
NA -1962	NOR-1978	SPX-1991	MXF-1994
PI -1974	CIP-1983		

Table 2: Resistance of *Escherichia* versus Resistance of *Klebsiella* the most prevalent genera

QUIN	*ESCHERICHIA* (n=108)		*KLEBSIELLA* (n=99)		P-value
	Resistant	%	Resistant	%	
NA	41	38.0	38	38.4	0.95
PI	41	38.0	42	42.4	0.51
NOR	33	30.6	34	34.3	0.56
CIP	32	29.6	33	33.3	0.57
SPX	32	29.6	36	36.4	0.30
MXF	32	29.6	36	36.4	0.30
Resistant to all	27	25	31	31.3	0.31

N: number of isolates, NA: nalidixic acid, PI: pipemidic acid, NOR: norfloxacin, CIP: ciprofloxacin SPX: sparmofloxacin, MXF: moxifloxacin

Table 3: Cross-Resistance to the quinolones/fluoroquinolones

ANTIBIOTIC	Nalidixic acid	Pipemidic acid	Norfloxacin	Ciprofloxacin	Sparfloxacin	Moxifloxacin
Nalidixic acid	-	**98.1%**	81.7%	77.9%	83.7%	83.7%
Pipemidic acid	89.5 %	-	75.44%	71.9%	78%	78.07%
Norfloxacin	**98.8%**	**100%**	-	95.4%	**98.84%**	**98.8%**
Ciprofloxacin	96.4%	97.6%	97.6%	-	**100%**	**98.8%**
Sparfloxacin	94.6%	96.74	92.4%	91.3%	-	**100%**
Moxifloxacin	94.57%	96.74%	92.4%	91.3%	**100%**	-

DISCUSSION

In this study we found out that the resistance of the enterobacteriaceae species to quinolone was high. Six quinolones belonging to the first four generations were selected for testing in this study. We found that the isolates had highest resistance to a first generation quinolone, pipemidic acid, whereas the lowest resistance was in ciprofloxacin, a second generation quinolone.

Pipemidic acid (first generation) was seen to be the least potent of the selected quinolones (figure 1). However, there was no significant difference in rates of resistance between the use of pipemidc acid and nalidixic acid the other first generation quinolone assessed. This is similar to studies [11, 13]. The potency peaks at ciprofloxacin a second generation quinolone (Figure 1). There was no statistically significant difference between the resistance observed with cipfloxacin and norfloxacin, all of second generation and that observed between Ciprofloxacin and the third and fourth generations (Sparmoloxacin and moxifloxacin). This high potency of ciprofloxacin was also reported in Nigeria [13]

However, the difference in resistance was statistical significance between the first generation quinolones (Nalidixic acid and pipermidic acid) and the two second generation FQ (Norfloxacin and ciprofloxacin). There was also a significant difference in potency between the first generation and the third and fourth generations (P-Values<0.05). This significant difference was also reported in other studies [13, 14].

This change in resistance in the generations was explained by Namboodiri and others as that, resistance in the quinolones often emerges at low-levels by acquisition of an initial resistance-conferring mutation or gene. Acquisition of subsequent mutations leads to higher levels of resistance to the first generation quinolones, nalidixic acid and a broadening of the resistance spectrum to include second generation quinolones (ciprofloxacin and norfloxacin) followed by newer third and fourth generations (sparmofloxacin and Moxifloxacin) [15].

The rate of cross resistance was high. It has also been reported that cross resistance between the older quinolones and fluoroquninolone exist in human as well as vetinary medicine and that the mechanism responsible for the resistance is similar in both situations [11].

The most resistant species was *Klebsiella* (Table I). This high resistant rate in *Kebsiella* sp was also observed in Greece, Nigeria, and United Kingdom [13, 17, 18]. Similarly, high level of resistance was also found in *Enterobacter* species as reported in previous studies. Several factors may explain the high prevalence of resistance in *Klebsiella sp* and *enterobacter sp.* Most importantly, these are hospital pathogens which cause nosocomial infections [5, 18]. Very low resistance was observed in *Salmonella* isolate which confirmed the fact that high-level of quinolone resistance is relatively uncommon in *Salmonella* spp. [11, 19] (Hopkins et al., 2005, Chen et al., 2011). The difference in resistance between the most prevalent species, *Escherichia* and *klebsiella*, was not statistically significant with p-value =0.31 (Table 2) however, *Klebsiella* was the more resistant of both species.

In some countries such as China, quinolones are commonly used for disease prevention and poultry production. The prudent use of quinolones is thus necessary in veterinary medicine to minimize the spread of resistant strains [11, 20].
Continued overuse of these antimicrobials in clinical medicine can promote resistance and is likely to limit the effectiveness of the quinolones. Also overuse of a single agent will ultimately result in resistance in the entire class [21]

Our study had some limitations. We carried out susceptibility testing only on one third and one fourth generation quinolone. Also our sample size was limited to only 300 isolates. We therefore recommend further studies with a larger number of isolates and more Qs and FQs

CONCLUSION

This study has shown that overall there is no significant difference in the susceptibility between the Qs of the first generation and the FQs in the treatment of enterobacterial infections. The high of percentage quinolone resistance makes it necessary for us to use a rational in prescribing these drugs.

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CONFLICT OF INTEREST

Authors declare they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

L.E.E, T.M, and O.A.M-C designed the study. L.E.E, TM, JA, G.H.K, M.M, E.A, I. G and LEE drafted the first version. All authors participated in the final version.

REFERENCES

[1] **Lennete, E. H., Balows A., Hausler Jr. W. J., and Shadomy H. J.,** (1985). Manual of clinical microbiology, fourth edition. Washington D. C: American Association of Microbiology Press. 1985; 263-277.

[2] *Shah. A. A., F. Hasan and A. Hameed*. 2002. **Study on the prevalence of enterobacteriacae in hospital acquired and community acquired infections.** Pakistan J. Med. Res. 41(1)

[3] **Bager F. and Helmuth R.** (2001). Epidemiology of resistance to quinolones in *Salmonella Vet. Res*. 32: 285–290

[4] **Minh Vien L. T., Baker S., Thao L. T. P., Tu L. T.P., Thuy C. T., Nga T. T. T., Minh Hoang N. V. M., Campbell J. I., Yen L. M., Hieu N. T., Chau N. V. V., Farrar J., and Schultsz C**. (2009). High prevalence of plasmid-mediated quinolone resistance determinants in commensal members of the enterobacteriaceae in Ho Chi Minh City, Vietnam. *Journal of Medical Microbiology*. **58**; 1585-1892

[5] **Livermore, D. M., James D., Reacher M., Graham C., Nichols T., Stephens P., Johnson A. P., and George R. C.,.** (2002). Trends in fluoroquinolones Resistance in Enterobacteriaceae from Bacteremias, England and Wales, 1990-1999 Ciprofloxacin Research. *Emerging Infectious Diseases*. **8**(5): 473-478

[6] **Larouche G.** (2001). Les Quinolone: Des Annees Soixante á Aujourd'hui. *Pharmatuel* **34**(2): 40-46

[7] **Eric Scholar M.** (2002). Fluoroquinolones: Past, Present, and Future of Novel Group of Antimicrobial Agents. *American Journal of Pharmaceutical Education*, Summer 2002

[8] **Kerr K.** G. (2004). Quinolone Antimicrobial Agent 3rd Ed. *Journal of Clinical Pathology***. 57;** 896-902

[9] **Lautenbach E., Strom B., L, Nachamkin I., Bilker W. B., Marr A. M., Lori A. Larosa, and Neil O. F.,** (2004). Longitudinal Trends in Fluoroquinolone Resistance among Enterobacteriaceae Isolates from Inpatients and Outpatients, 1989–2000: Differences in the Emergence and Epidemiology of Resistance across Organisms. *Clinical Infectious Diseases***. 38:**655–62

[10] **Leistevuo T., Toivonen P, Osterblad M., Kuistila M., Kahra A., Lehtonen A., and Huovinen P**. (1996). Problem of antimicrobial resistance of fecal aerobic Gram-Negative Bacilli in Elderly. *Antimicrobial agents and chemotherapy*.**40**(10): 2399-2403

[11] **Chen X., Pan W., Zhang W., Pan Z., Gao S., and Jiao X.,** (2011). Quinolone resistance in Escherichia coli and Salmonella spp. Isolates from diseased chickens during 1993-2008. *African Journal of Microbiology Research* Vol. 5(19) 3078-3083

[12] **Clincal Laboratory Standard Institute** (2007). Performance Standards For Antimicrobial Susceptibility Testing, 17th Informational Supplement, M100-S17. Wayne, PA: Clinical and Laboratory Standards Institute.

[13] **Momoh A.R., Odike M.A.C., Olowo S., Momoh A.A. Okolo. (**2007). Resistance pattern of Urinary tract infection Bacterial Isolate to selected quinolones. *African Journals Online*. **9**(12)22

[14] **Horhat F, Muntean D., Hogea E., Horhat D., Craciunescu M., Licker M., Rosca A.,, Baditoiu L. M., Moldovan R.,**. (2010). Quinolone Resistant Enterobacteriaceae Strains Isolated from Urinary Tract Infections in the Intensive Care Unit. *Journal of Experimental Medical & Surgical Research.* **4**: 308 – 310

[15] **Namboodiri S. S., Opintan J. A., Lijek R. S., Newman M. J and Okeke I. N.** (2011). Quinolone Resistance in *Escherichia coli* from Accra Ghana. *BMC Microbiology* 11:44 doi:10.1186/1471-2180-11-44

[16] **Piddock LJ., Hall MC., Walters RN.,** (1991). Phenotypic Characterization of quinolone-resistant mutants of enterobacteriacece selected from wild type, gyr A and multiple-resistant (mar A) type strains**.** *J. Antimicrob. Chemoter*. **28**(2):185-98

[17] **Skandami-Epitropaki V., Xanthaki A., Tsiringa A. Fotiou P., Kontou CHA, Toutoua M.** (2008). Fluoroquinolones resistance in Enterobacteriaceae strains isolated from community acquired urinary tract infections. *European Society of Clinical Microbiology and Infectious Diseases.* 2008; 8.

[18] **Toukam M., Lyonga E. E., Assoumou M.C. O., Fokunang C. N., Atashili J., Kechia A. F., Gonsu H. K., Mesembe M., Eyoh A., Ikomey G., Akongnwi E., Ndumbe P.** (2010). Quinolone and fluorquinolone resistance in Enterobacteriaceae isolated from hospitalised and community patients in Cameroon. *Journal of Medicine and Medical Sciences.* 1(10): 490-494.

[19] **Hopkins KL, Davis RH., Threlf fall EJ**. (2005). Mechanisms of Quinolone Resistance in *E. coli* and *Salmonella:* Recent developments. *Int. J. Antimicrobial Ag.,* **25**: 358-373

[20] **Bazile-Pham-Khac S., Truong Q. C., Lafont J-P., Gutmann L., Zhou X. Y., Osman M. and Moreau N.J.** 1996. Resistance to Fluoroquinolones in *Escherichia coli* Isolated from Poultry. *Antimicrobial agents and chemotherapy*. **40**(6): 1504-1507

[21] **Gallini A.**, **Degris E., Desplas M., Bourrel R., Archambaud M., Montastruc J-L., Lapeyre-Mestrs M. and Sommet**. (2010). Influence of fluoroquinolone consumption in inpatients and outpatients on ciprofloxacin-resistant Escherichia coli in a University Hospital. *J of Antimicrobial Chemother*. **65**:2650-2657