Journal of Medicine and Medical Sciences Vol. 1(10) pp. 490-494 November 2010
Available online http://www.interesjournals.org/JMMS
Copyright ©2010 International Research Journals

Full Length Research Paper

Quinolone and fluoroquinolone resistance in Enterobacteriaceae isolated from hospitalised and community patients in Cameroon

Toukam M[1], Lyonga E.E[1], Assoumou M.C.O[1], Fokunang C.N[1*], Atashili J[2], Kechia A.F[1], Gonsu H.K[1] Mesembe M[1], Eyoh A[1], Ikomey G[1], Akongnwi E[1], Ndumbe P[1, 2]

[1]Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon.
[2] Faculty of Health Sciences, University Of Buea, Cameroon.

Accepted 19 September, 2010

Quinolones and fluoroquinolones are frequently used for the presumptive treatment of suspected enterobacterial infections in Cameroon and other resource-limited settings. This study aimed at describing patterns of resistance to quinolones and fluoroquinolones (QFR) in Enterobacteriaceae and thus allow for a better management of patients in these settings. During a 10-month period, a total of 300 enterobacterial strains were isolated from 13 different clinical specimens from hospitalized patients (HP) and community patients (CP). Identification was done using the API 20E. The sensitivity to antibiotics was tested using Kirby-Bauer disk diffusion method according to Clinical Lab. Standard Institute criteria. Out of the 300 isolates identified as Enterobacteriaceae, 58% were from HP while 42% were from CP. The prevalence of each genus was: *Escherichia* 36%, *Klebsiella* 33%; *Enterobacter* 8%, *Proteus* 8% and others 15%. QFR was detected in 25.7% of all isolates, with a significantly higher prevalence in HP (31.8%) compared to CP (17.3%), p-value=0.0069. Genus-specific resistance rates in HP and CP were respectively: *Escherichia* 33.3% and 16.7%; *Klebsiella* 39.7% and 16.7%; *Enterobacter* 25% and 37.5%; *Proteus* 9% and 7.7%. Resistance to pipemidic acid and ciprofloxacin was present in 38% and 28% of all isolates respectively. Resistance to more than one quinolone/fluoroquinolone was observed in 36.7% of isolates. QFR resistance was high in this population of Enterobacteriaceae isolates from Cameroon. Resistance was highest in hospital patients and in *Klebsiella* isolates. Guidelines for presumptive treatment should be implemented for this resistance pattern.

Keywords: Enterobacteriaceae, quinolone, fluoroquinolone, resistance, community patience, Cameroon

INTRODUCTION

Enterobacteriaceae species are incriminated in virtually any type of infectious disease and can be recovered from any specimen received in the laboratory. Immunocompromised patients or debilitated patients are highly susceptible to hospital acquired enterobacterial infections (Abraham et al., 1981; Edwin et al., 1885; Elmer et al., 1992; Xilin et al., 2006). These infections are often treated with quinolones and fluoroquinolones. The appearance of quinolone and fluoroquinolones resistant bacteria was observed immediately after the introduction of these antimicrobials into clinical practice to treat infections in hospitalized and community acquired infections (Betty et al., 1998; Hedi et al., 2005; Carmen et al., 2008).

It has been demonstrated that the trend in quinolone and fluoroquinolones resistance has been increasing over the years. A study carried out in England and Wales shows that the prevalence of resistance of *Klebsiella* species to fluoroquinolones rose from 3.5% in 1990 to 9.5% in 1996 (Antti et al., 1999; David et al.,2002; Api, 2006). Another study carried out in Greece from 2005 to 2007 revealed the overall resistance of Enterobacteriaceae to be 15.6% with 21.1% in Hospitalized patients and 6.2% in community patients (David et al., 2001; Kerr, 2004; Skandami-Epitropaki et al.,2008). Therefore knowledge of the local prevalence of pathogens and their antimicrobial sensitivity patterns is essential for clinicians in their routine work (Arjana et al., 2002; Karel et al., 2005; CLSI, 2006). This study was aimed at establishing Enterobacteriaceae resistance

*Corresponding author email: charlesfokunang@yahoo.co.uk; Tel: +237 94218670 ; Fax : +237 2231 27 33

patterns to quinolones and fluoroquinolones in view of implementing a better control strategy for the care of patients particularly in situations were Enterobacteriaceae infections are suspected but antimicrobial susceptibility testing cannot be done.

METHODOLOGY

A cross- sectional descriptive study was carried out with a sample size of 300. The ten month study period spanned from December 2006 - September 2007. Specimens were collected from 13 different clinical specimens from hospitalised and community patients at the Yaoundé General Hospital. These specimens were cultured in Eosin methylene Blue Agar incubated for 18-24 hours at 37°C. Isolated colonies were gram stained using the standard laboratory culture procedures. The gram-negative bacilli were then identified using the Api 20 identification kits (BioMérieux SA, Lyon, France). Antimicrobial susceptibility testing was then carried out on all species identified from the Enterobacteriaceae family including the quality control strain ATCC 25922. The Kirby-Bauer disc diffusion method was used for susceptibility testing (Betty et al., 1998; CLSI, 2006). The antibiotics tested for susceptibility included two quinolones (Nalidixic acid and pipemedic acid) and four fluoroquinolones (norfloxacin, ciprofloxacin, sparmofloxacin and moxifloxacin). The diameters of the zones of inhibitions were then measured. The susceptibility (sensitive, intermediate, or resistance) was determined using the Clinical and Laboratory Standard Institute (CLSI) Performance standards for Antimicrobial Susceptibility testing (David et al., 2001; Armand et al., 2006).

RESULTS

300 isolates were identified as Enterobacteriaceae and distributed into different taxonomic groups as shown on table 1

29 different species were identified from eleven genera of the Enterobacteriaceae family. The eleven genera were grouped into 5 main Tribes of the family; Escherichiaeae, Klebsielleae, Proteeae, Salmonelleae, and Citrobactereae. 36% of the isolates were *Escherichia species*; 33% were *Klebsiella* species; *Enterobacter* and *Proteus* both had 8% (24/300) each; *Serratia* 7% *Salmonella* 3%); *Kluyvera, Pantoea*, and *Morganella* each had 0.7% of the isolates while *Shigella* had 0.3% 25.7% of the Enterobacteriaceae were found to be resistant to all six quinolones. 31.3% of *Klebsiella* isolates were resistant; *Enterobacter* 29.2%; and *Escherichia* 26.9%. The highest percentage of resistance 38% was observed in pipemidic acid while the least resistance was observed in ciprofloxacin.

58% of the isolates were from hospitalized patients (HP) and 42%) were from community patients (CP). 31.8% of these isolates from HP were resistant to all six quinolones and 17.3% of isolates from the CP were resistant to all quinolones (p-value=0.0069) as shown on Table 2

Klebsiella showed the highest level of resistance to HP with mean value of 39.7% with *Proteus* recording the least resistance of 9%. For CP resistance out of 127 samples *Escherichia and Klebsiella genus* showed the highest resistance with mean value of 16.7%, while the *proteus* showed the least resistance to CP with a mean value of 7.7% (Table 3)

DISCUSSION

A total of 300 strains of Enterobacteriaceae were isolated for this study. The isolates were from patients aged 0-97 years and from 13 different types of specimens; bone fragment, bed sore, cervical/vaginal swabs, hemoculture, pleural fluid, pus, seminal fluid, sputum, stool, urethral swab, urine, urinary catheter and wound. Members of the Enterobacteriaceae family may be incriminated in virtually any type of infectious disease and recovered from any specimen received in the laboratory (Elmer et al., 1992; Deguchi et al., 1997; Dana et al., 2000; David et al., 2002).

Majority (69%) of the Enterobacterial isolates were from urine specimens. This confirms the findings of Arslan and collaborator in Turkey in 2005 (Harold, 2002; James et al., 2002; Hande et al., 2005) that urinary tract infections (UTIs) are common infections; it is estimated that 150 million UTIs occur yearly worldwide, resulting to 6 billion dollar spending in direct healthcare cost. Specimens like bed sore, bone fragment, pleural fluids, seminal fluid and wound are rare specimens in most clinical microbiology laboratories. Therefore, each accounted for only 0.3% of the isolates.

Of the 27 genera and 100 species of Enterobacteriaceae described by Farmer et al at the Centers for Diseases Control (CDC) in 1991 (Edwin et al., 1985; Scholar, 2002; Caliopsia, 2006), 29 pathogenic species were identified in this study from eleven of these genera using the Api 20E identification system. *E. coli* (36%) was the bacterial species most commonly isolated in laboratories and has been incriminated in infectious diseases involving virtually every human tissue and organ (Geo et al., 1991; Elmer et al., 1992; Oliphant, 2002). This was confirmed as being the most prevalent as observed by Skandami-Epitropaki and collaborators in Greece (Brisse et al., 2000; David et al., 2002; Scholar, 2002; Skandami-Epitropaki et al., 2008. Similar studies of E.coli as the most commonly isolated bacteria of the Enterobacteriaceae family was earlier reported by Livermore and his research team in England and Wales (Tina et al., 1996; Brisse et al., 2000; Caliopsia, 2006).

Klebsiella species (33%) ranked second in prevalence like in previous studies (Genevieve, 2001; Pieboji et al., 2004; Api, 2006). Species like *Morganella, Kluyvera* and *Pantoea* which are normally not pathogenic but become pathogenic in immunosuppressed individuals each accounted for 0.7% of the isolates. The trend recorded of most prevalent; *Escherichia coli, Klebsiella, Enterobacter*

Table 1. Prevalence of the Enterobacteriaceae isolates by Tribe, genus and species

TRIBE	GENUS	N°	%	SPECIES	N°	
Escherichieae	*Escherichia*	108	36%	*Coli*	108	36 %
	Shigella	1	0.3 %	*Species*	1	0.3 %
Klebsielleae	*Enterobacter*	24	8%	*Aerogenes*	3	1 %
				Asburiae	2	0.7 %
				Cloacae	17	5.7 %
				sakazakii	2	0.7 %
	Pantoea	2	0.7%	*Spp 2*	1	0.3%
				Spp4	1	0.3%
	Klebsiella	99	33 %	*pneumoniae*	86	28.7 %
				Ornithinolytica	5	1.7 %
				oxytoca	8	2.7 %
	Serratia	21	7 %	*marcescens*	7	2.3 %
				Ficaria	1	0.3 %
				Fonticola	1	0.3 %
				Odonifera 1	7	2.3 %
				Odonifera 2	3	1 %
				plymuthica	1	0.3 %
				Rubidiae	1	0.3 %
	Morganella	2	0.7 %	*Morganii*	2	0.7 %
Proteeae	*Proteus*	24	8%	*Mirabilis*	23	7.7 %
				Vulgaris	1	0.3 %
Salmonelleae	*Salmonella*	9	3%	*Paratyphi*	1	0.3 %
				Typhi	1	0.3 %
				spp	7	2.3 %
Citrobactereae	*Citrobacter*	8	2.7 %	*Braakii*	3	1 %
				Freundii	2	0.7 %
				Koseri	2	0.7 %
				youngae	1	0.3 %
other	*Kluyvera*	2	0.7%	*Spp*	2	0.7%
TOTAL		**300**	**100%**		**300**	**100%**

Table 2. Genera isolated in hospitalized versus Community patients

GENUS	Hospitalized Patients (n=173)		Community Patients (n=127)	
	N°	%	N°	%
Klebsiella	63	36.4%	36	28.3
Escherichia	54	31.2%	54	42.5%
Enterobacter	16	9.2%	8	6.3%
Serratia	15	8.7%	6	4.9%
Proteus	11	6.4%	13	10.2%
Salmonella	7	4.1%	2	1.6%
Citrobacter	3	1.7%	5	3.8%
Pantoea	2	1.2%	0	0
Kluyvera	1	0.6%	1	0.8%
Morganella	1	0.6%	1	0.8%
Shigella	0	0	1	0.8%
Total	**173**	**100%**	**127**	**100%**

Table 3. Resistance of hospitalised (HP) versus community patients (CP) to all Q and FQ used

GENUS	HP (N=173)	CP (N=127)	P-VALUE
Klebsiella	25 (39.7%)	6 (16.7%)	
Escherichia	18 (33.3%)	9 (16.7%)	
Enterobacter	4 (25%)	3(37.5)	
Serratia	3 (20%)	3(50%)	
Proteus	1 (9%)	1(7.7%)	
Others	4(28.8%)	0 %	
Total	55 (31.8%)	22(17.3%)	0.0069

and *Proteus* have also been reported (Pieboji et al., 2004; Api, 2006).

Overall, of the 300 Enterobacteriaceae isolates studied a resistance rate of 25.7% (resistant to all six antibiotics) was observed which was higher than the 15.6% observed by Skandami-Epitropaki et al (2008). It was also observed in this study that a high percentage, (37.7%) of the isolates were resistant to at least one of the quinolones. Many factors may account for these high resistant rates such as the excessive use of antibiotics and the availability of generic drugs of very broad spectrum.

Klebsiella species were the most resistant strains with an overall resistance of 31.3%. This was closely followed by *Enterobacter* species 29.2 % and thirdly *Escherichia* species 25%. One factor which may explain the greater prevalence of resistance in *Klebsiella* and *Enterobacter* species is the fact that *Klebsiella* and *Enterobacter* are primarily Hospital pathogens (nosocomial infections), these tend to be resistant.

The resistance of all Enterobacteriaceae for hospitalized patients (HP) was 31.8% and 17.3% for community patients (CP). This was higher than the 21.1% obtained for HP and 6.2% for CP (Skandami-Epitropaki et al., 2008). The overall resistance in hospitalised patients was significantly higher than that in community patients ($p<0.05$). The fact that most HP patients tend to have chronic infections may have accounted for this difference.

The prevalence of resistance for *Klebsiella* in HP was 39.7% and 16.7% in CP this also was again higher than the values observed by Skandami-Epitropaki et al (2008). The resistance for *E. coli* was 33.3% for HP and 16.7% for CP whereas a previous study carried out showed a resistance of 17.4% for HP and 5.05% for CP (Kerr, 2004; Karel et al., 2005). 56.3% Isolates from urinary catheter were resistant. This may be due to the prolonged use of urinary catheter and poor aseptic handling procedures which predispose the patients to infection with hospital acquired pathogens that are very resistant.

Many factors may have contributed to such high rates of resistance, including; presumptive treatment without antimicrobial susceptibility testing, misuse of antibiotics by health professionals, unskilled practitioners and public (antibiotics can be purchased without prescription), poor drug quality, unhygienic conditions accounting for the spread of resistant bacteria and inadequate surveillance as cited by Gangoue Pieboji et al in Cameroon (Deguchi et al., 1997; Genevieve, 2001; Oliphant, 2002). The easy access to antibiotics in some community pharmacies without prescription has led to patients' vulnerability to excessive use of antibiotics in Cameroon. This has become a very big health concern issues the health service is trying to sensitise the public and develop strategies for control. Antimicrobial resistance often leads to therapeutic failure of empirical therapy; therefore knowledge of the local prevalence of pathogens and their antimicrobial sensitivity patterns is essential for clinicians in their routine work (Arjana et al., 2002, Aurora et al., 2004, Xilin et al., 2006).

CONCLUSION

Quinolone and fluoroquinolone resistance was high in this population of Enterobacteriaceae isolates from Cameroon. Resistance was highest in hospital patients and in *Klebsiella* isolates. Guidelines for presumptive treatment should be implemented for this resistance pattern.

REFERENCES

Abraham IB, Charles D, Joshua F (1981). Medical microbiology and infectious diseases. London: W.B. Saunders Company. pp. 340-351.

Antti H, Siitonen A, Kotilanen P, Huovinen P (1999). Increasing Fluoroquinolones Resistance in *Salmonella* serotypes in Finland during 1995-1997. J. Antimicrobial. Chemother. 43:145-148.

Api E (2006). Identification system for Enterobacteriaeae and other no-fastidious gram-negative rods. Lyon: BioMérieux SA.

Arjana TA, Tera T, Kalenic S, Jankovic V (2002). Surveillance for Antimicrobial Resistance in Croatia. Emerg. Infect. Dis. 8:1-6.

Armand P, Fluit AD, Verhoef J, Maurine A. Leverstein-van H (2006). Enterobacter cloacae outbreak and emergence of quinolone resistance gene in Dutch hospital. Emerg. Infect. Dis. 20: 12-16.

Aurora E, Pop-Vicas S, Erika MC, D'Agata P (2004). The Rising Influx of multidrug-resistant Gram-Negative Bacilli into a Tertiary Care

Hospital. Clin. Infect. Dis. 40:1792-1798.
Betty AF, Daniel F, Sahm T, Alice S, Weissfeld. (1998). Bailey and Scott's Diagnostic Microbiology. Tenth Edition. Mosby. Inc; p.p 120-126.
Brisse S, Milatovic D, Fluit AC, Verhoef J, Schmitz FJ (2000). Epidemiology of Quinolone Resistance of *Klebsiella Pneumoniae and Klebsiella oxytoca* in Europe. European .J. Clin. Microbiol. Infect. Dis. 19:64-68.
Caliopsia F (2006). Sensitivity to antibiotics of *Escherichia coli* strains from children admitted to the "Sf. Loan" Clinical emergency hospital for children in Galati during 2006. Bacteriol. Virusol. Parazitol. Epidemiol. 52: 37-44.
Carmen AI, Ana CG, Maria CBT, Munerato P, Libera MDC (2008). Quinolone-Resistant *Escherichia coli*. *The* Brazilian. J. Infec. Dis. 12**:**5-9.
Clinical Laboratory Standard Institute (CLSI) (2006). Performance standards for Antimicrobial Susceptibility testing Sixteenth informational supplement. 26: M100-S16 Wayne, Pensylvania. USA.
Dana E, King R, Malone A, Sandra L. (2000). New classification and update on the quinolone antibiotics. Am. Fam. Phys. 61:2741-2748.
David M, Livermore, P, Dorothy J, Reacher M, Catriona G, Thomas N, Stephens P (2002)**.** Trends in fluoroquinolones Resistance in Enterobacteriaceae from Bacteremias, England and Wales, 1990-1999 Ciprofloxacin Research. Emerg. Infect. Dis. 5:35-40.
David T, Bearden L, Larry H (2006). Danziger**.** Mechanism of Action and Resistance to Quinolones. Pharmacother. 21:224-232.
Deguchi T, Kawamura T Yasuda M (1997). In vivo selection of *Klebsiella pneumonia* enhanced quinolone resistance during fluorquinolone treatment of urinary tract infections. J. Antimicrob. Chemother 41:1609-161.
Edwin H, Lennete A, Balows WJ, Hausler JR, Shadomy HJ (1985). Manual of clinical microbiology, fourth edition. Washington D. C: American Assoc. Microbiol. Press. Pp. 263-277.
Elmer WK, Sephen D, William MJ, Paul C, Schreekenberger P, Washington C. Winn Jr (1992). Introduction to Diagnostic Microbiology. Philadelphia: JB. Lippingcott Company. Pp. 41-73.
Genvième L (2001). Les quinolones: Des années soixante à aujourd'hui Pharmacotherapie théorique. Pharmactuel 34: 40-46.
Geo F, Brooks J, Butel L, Nicolas OE, Jawetz L, Joseph M, Edward A (1991).Adelberg's Medical Microbiology. Nineteenth Edition. California: Appleton and Lange Press. Pp. 212 – 228.
Hande A, Ozem KA, Onder E, Funda T (2005). Risk factors for ciprofloxacin resistance among Escherichia coli strains from community-acquired urinary tract infection in Turkey. J. Antimicrob. Chemother. 56: 914-918.
Harold JB (2002). Microbiological Apllication. Eighth Edition. New York: McGraw Hill Company pp. 41-44.
Hedi M, Mammeri MV, Laurent P, Martinez-Martinez L, Patrice N (2005)**.** Emergence of Plamid-Mediated quinolone resistance in *Echerichia coli* in Europe. Antimicrob. Agents Chemother. 49: 71-76.
James A, Karlowsky L Kelly J, Thornsberry L, Mark FS (2002). Trends in Antimicrobial Resistance among Urinary Tract Infections of *Escherichia coli* from female outpatients in United State. Antimicrob. Agent. Chemother. 46:2540-2545.
Karel U, Milan K, Jan S, Dagmar K (2005). Utilization of fluoroquinolones and Escherichia coli resistance in urinary tract infection: inpatients and outpatients. Pharmacol. Drug Saf. 14:10-16
Kerr KG (2004). Quinolone antimicrobial agents. J.Clin. Pathol. 57:894-896.
Oliphant CM, Gary MG, Permanente K (2002). Quinolones: A Comprehensive Review. American. Fam. Phys. 65:455-4
Pieboji JG, Koulla-Shiro S, Ngassam P, Adiogo D, Ndumbe P (2004). Antimicrobial Resistance of Gram-Negative Bacilli from Inpatient and Outpatients at the Yaounde Central Hospital, Cameroon. International. J. Infect. Dis. 8**:**147-154.
Scholar EM (2002). Fluoroquinolones: Past, Present and future of a novel group of antibacterial agents. American. J. Parmaceut. Educ. 16:14-22.
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. Soc. Clin. Microbiol. Infect. Dis. 8: 26-32.
Tina L, Toivonen P, Osterblad M, Kuistila A, Kahra A (1996). Problem of Antimicrobial Resistance of Faecal Aerobic Gram-Negative Bacilli in the Elderly. Antimicrob. Agents. Chemother. 40:2399-2403
Xilin Z, Muhammad M, Nymph C (2006). Lethal Action of quinolones against a Temperature-sensitive dnaB Replication mutant of *Escherichia coli*. Antimicrob. Agent Chemother. 50**:**:362-364.