Evaluation of Mutant Prevention Concentrations of Cephalosporin antibiotics for fecal *Escherichia coli* isolated in Keffi, Nigeria

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ABSTRACT

Mutant Prevention Concentration (MPC) concept provides *in vitro* justification of a dosing regimen of antimicrobial agent that can prevent the emergence of least susceptible resistant mutant. This study evaluates the MPC of cephalexin, cefuroxime, ceftazidime and cefepime under two different temperature conditions for *E. coli* isolated from stool of patients. The study also investigates effect of temperature on the selection of resistance mutants. A total of fifty (50) isolates of *E. coli* was isolated and identified by standard procedure from stool of patients attending Nasarawa State University Health Centre Keffi, Nigeria. MPCs and mutant recovery were determined at 37°C and 41°C by standard methods. MPC at both 37°C and 41°C were the same for each of the drugs. MPCs for 50% of the isolates (MPC_{50}) were: cephalexin (421.4 µg/ml[6.2 x MIC]), cefuroxime (209.0 µg/ml[3.3 x MIC]), ceftazidime (81.0 µg/ml[2.2 x MIC]) and cefepime (30.6 µg/ml[1.4 x MIC]); MPC for 90% of the isolates (MPC_{90}) were: cephalexin (471.8 µg/ml[3.9 x MIC]), cefuroxime (465.0 µg/ml[3.8 x MIC]), ceftazidime (454.8 µg/ml[4 x MIC]) and cefepime (438.4 µg/ml[7.2 x MIC]). The MPC_{50}/MIC_{50} ratios at both 37°C and 41°C were in the order: cephalexin>cefuroxime>ceftazidime>cefepime; but MPC_{90}/MIC_{90} ratios were in the reverse order. The mutant recovery at MPC_{50} at both 37°C and 41°C were insignificantly

different (p>0.05) for cephalexin (p=0.5918), cefuroxime (p=0.6335), ceftazidime (p=0.2318) and cefepime (p=0.0862); mutant recovery at MPC_{90} was also insignificantly different (p>0.05) for cephalexin (p=0.8075), cefuroxime (p=0.396), ceftazidime (p=0.4975) and cefepime (p=0.1548). Mutant recovery at MPC_{50} of all cephalosporins differ significantly (p=0.0430: p<0.05) from each other at 37°C but not at 41°C (p=0.0973: p>0.05); mutant recovery at MPC_{90} of all cephalosporins were insignificantly (p>0.05) different both at 37°C (p=0.1616) and 41°C (p=0.2633). *Escherichia coli* develop resistance mutation less likely with higher than lower generations of cephalosporin antibiotics. Temperature has no effect on the prevention of selection and enrichment of resistance mutants, but enhances the extent of recovery of mutants justifying the common practice of administrating high dose of antimicrobial agent at high body temperature during therapy of bacterial disease.

Key Words: Mutant Prevention Concentration; *Escherichia coli*; Cephalosporin

Running Title: MPC of Cephalosporin for fecal *E. coli* in Keffi

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Introduction

Escherichia coli infections are usually treated with antimicrobial agents; but the success of therapy is limited by the development of resistance mechanisms (Todar, 2007). Traditional dosing of antimicrobials is based on the antibiotic minimum inhibitory concentration (MIC), which block the growth of the majority of susceptible pathogens, but selectively enrich the

resistant mutant portion of the population (Drlica & Zhao, 2007; Roberts et al., 2008; Credito et al., 2010). Focus on killing susceptible cells overlooks resistant mutant subpopulations that may be present before treatment or generated during therapy. Consequently, resistance can emerge during the eradication of susceptible cells. Optimization of dosing strategies (so-called anti-mutant dosing) has been advocated as a way to limit antimicrobial resistance (Liang et al., 2011). This employs a therapeutic drug concentration, called 'mutant prevention concentration' (MPC) (Dong et al., 1999), which prevents the growth of the least susceptible single-step mutant present in a large bacterial population. MPC can be employed to compare the power of different antimicrobial agents to prevent emergence of antimicrobial resistance (Drlica, 2001).

Cephalosporins are among the many classes of antibiotics which may be used to treat *E. coli* infections (Salyers *et al.*, 2004; Todar, 2007). Resistance in *E. coli* isolates to cephalosporins is increasingly reported (Akins et al., 2002; Forward et al., 2004; Johnson et al., 2007; Thokar et al., 2010; Drawz & Bonomo, 2010). There is thus the need to explore anti-mutant dosing strategies using the MPC concept as a means to limit the development of resistance to cephalosporins. This study evaluates the MPC of cephalexin, cefuroxime, ceftazidime and cefepime for *E. coli* isolated from stool of patients in Keffi. The study also investigates the effect of a rise in temperature on both the cephalosporin antibiotic MPC values and mutant recovery.

Materials and Methods

Bacterial Isolates

A total of 50 fecal *E. coli* isolates were used in this study. They were isolated and identified from stool of patients attending Nasarawa State University Keffi Health Center using standard

cultural, microscopical and biochemical procedures (Cheesbrough, 2000). Pink colonies on MacConkey agar (BIOTEC Laboratories Ltd., Ipswich, United Kingdom) that grew with greenish metallic sheen characteristics on eosin methylene blue agar (BIOTEC Laboratories Ltd., Ipswich, United Kingdom) and which were indole positive, methyl red positive, Voges-Proskauer negative and citrate negative were confirmed as *E. coli*. Bacteria were stored in the refrigerator at 4°C on nutrient agar (NA: Merck KGaA, Darmstadt, Germany) slants and reactivated by sub-culturing on MacConkey agar and used in experiments.

Antibiotics

The antibiotics used were cephalexin (Ranbaxy Laboratories Ltd, India), cefuroxime (Glaxo Smith-Kline, India), ceftazidime (Glaxo Smith-Kline, Italy) and cefepime (Bharat Parenterals Ltd., India). All antibiotics were purchased from the Pharmacy Department, Federal Medical Center, Keffi, Nigeria. The stock solutions were prepared in appropriate solvents in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2012).

Determination of Minimum Inhibitory Concentration (MIC)

The MICs of the antibiotics against the *E. coli* isolates and quality control strain (*E. coli* ATCC 25922) were determined in triplicate using the CLSI macro-broth dilution method (CLSI, 2012). An adjusted inoculum of the test organism was inoculated into Mueller-Hinton broth (MHB: BIOTEC Laboratories Ltd., Ipswich, United Kingdom) containing two-fold dilutions of an initial antibiotic solution so that each tube contained approximately 1 x 10^5 colony-forming units (CFU). Results were observed and registered after 24-h incubation at 37°C. MIC was defined as the lowest concentration that inhibited visible growth. Cumulative frequency curves of the antibiotic MICs of isolates were plotted and MICs for 50% (MIC_{50}) and for 90% (MIC_{90}) of isolates were then generated from the plots.

Determination of Mutant Prevention Concentration (MPC)

The MPCs were determined as described elsewhere (Randall et al., 2001) with modifications. Briefly, the tested micro-organisms were cultured in 50 ml of MHB and incubated for 24 h. Then, the suspension was centrifuged (at 4000 g for 10 min) and re-suspended in 10 ml of MHB to yield a concentration of 5 x 10^{10} cfu/10 ml. The inocula were further confirmed through the serial dilution and plating of 100 µl samples on drug-free medium. A series of Mueller-Hinton agar (Fluka Biochemical, Spain) plates containing known concentrations of the aminoglycoside antibiotics were then inoculated with 200 µl each of re-suspended *E. coli* culture (containing approx. 10^{10} cfu). The inoculated plates were incubated for 48 h at 37°C and 41°C, and then screened visually for growth, and colonies counted after the incubation. The MPC was taken as the lowest aminoglycoside concentration that prevents the growth of any mutant after 48 h incubation. All experiments were performed in triplicate. Cumulative frequency curves of the antibiotic MPCs for the isolates were plotted and the MPCs for 50% (MPC_{50}) and 90% (MPC_{90}) of isolates were then generated from the curves. The frequency at which resistant mutant were recovered was calculated as the number of mutants growing in the presence of antibiotic per ml divided by the inoculum density (1.0 x 10^{10}cfu).

Statistical Analyses

Mutant recovery for 50% of isolates (MR_{50}) and mutant recovery for 90% of isolates (MR_{90}) were compared at 37°C or 41°C and between temperatures by one way analysis of variance (ANOVA) using Smith Statistical Package (SSP), version 2.80 (by Gary Smith, Pomona College, Claremont, California). Significance of differences was determined at the 5% probability level (that is at P = 0.05).

Results

MICs of antibiotics

The minimum and maximum antibiotic MICs (in µg/ml) were (min: max): cephalexin (16.0:128.0), cefuroxime (8.0:128.0), ceftazidime (8.0:128.0) and cefepime (8.0:128.0) as shown in Table 1. The MIC_{50} and MIC_{90} of isolates at 37°C and 41°C generated from the cumulative frequency curves in Figure 1 are as shown in Table 1.

Table 1: Minimum Inhibitory Concentration of some cephalosporins for 50% and 90% of fecal *Escherichia coli* isolates at 37°C and 41°C

Antibiotics	MIC Ranges (µg/ml)	Minimum Inhibitory Concentration (µg/ml)	
		MIC_{50}	MIC_{90}
Cephalexin	16.0-128.0	68.0 (≥ 64.0)	68.0 (≥ 64.0)
Cefuroxime	8.0-128.0	63.6 (≤ 64.0)	63.6 (≤ 64.0)
Ceftazidime	8.0-128.0	37.2 (≥ 32.0)	37.2 (≥ 32.0)
Cefepime	8.0-128.0	21.3 (≥ 16.0)	21.3 (≥ 16.0)

MIC_{50} = **Minimum Inhibitory Concentration for 50% of isolates;** MIC_{90} = **Minimum Inhibitory Concentration for 90% of isolates.**

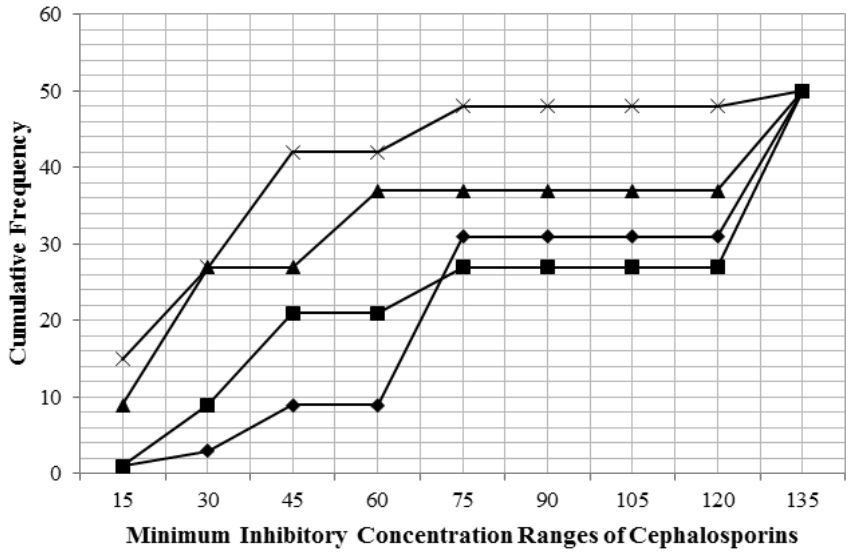

Figure 1: Cumulative Frequency Curves of cephalosporin MICs for isolates of *Escherichia coli* (◆ Cephalexin, ■ Cefuroxime, ▲ Ceftazidime, X Cefepime).

MPCs of antibiotics

The minimum and maximum antibiotic MPCs (in µg/ml) were (min: max): cephalexin (64.0:512.0), cefuroxime (16.0:512.0), ceftazidime (32.0:512.0) and cefepime (8.0:512.0) as shown in Table 2. The MPC_{50} and MPC_{90} of isolates at 37°C and 41°C generated from the cumulative frequency curves in Figure 2 are as shown in Table 2. The MPC_{50} and MPC_{90} for *E. coli* decreases with increasing generation of the cephalosporin; and are same at both 37°C and 41°C for each drug.

MPC/MIC ratio

The minimum and maximum antibiotic MPC/MIC ratios were (min: max): cephalexin (2.0:64.0), cefuroxime (1.0:32.0), ceftazidime (1.0:32.0) and cefepime (1.0:64.0) as shown in Table 3. The antibiotic MPC_{50}/MIC_{50} and MPC_{90}/MIC_{90} ratios for the isolates at 37°C and 41°C generated from the cumulative frequency curves in Figure 3 are as shown in Table 3.

Table 2: Mutant Prevention Concentration of some cephalosporins for 50% and 90% of fecal *Escherichia coli* isolates at 37°C and 41°C

Antibiotics	MPC Ranges (µg/ml)	Mutant Prevention Concentration (µg/ml)	
		MPC_{50}	MPC_{90}
Cephalexin	64.0 – 512.0	421.4 (≤ 512.0)	471.8 (≤ 512.0)
Cefuroxime	16.0 – 512.0	209.0 (≤ 256.0)	465.0 (≤ 512.0)
Ceftazidime	32.0 – 512.0	81.0 (≥ 64.0)	454.8 (≤ 512.0)
Cefepime	8.0 – 512.0	30.6 (≤ 32.0)	438.4 (≤ 512.0)

MPC_{50} = **Mutant Prevention Concentration for 50% of isolates;** MPC_{90} = **Mutant Prevention Concentration for 90% of isolates.**

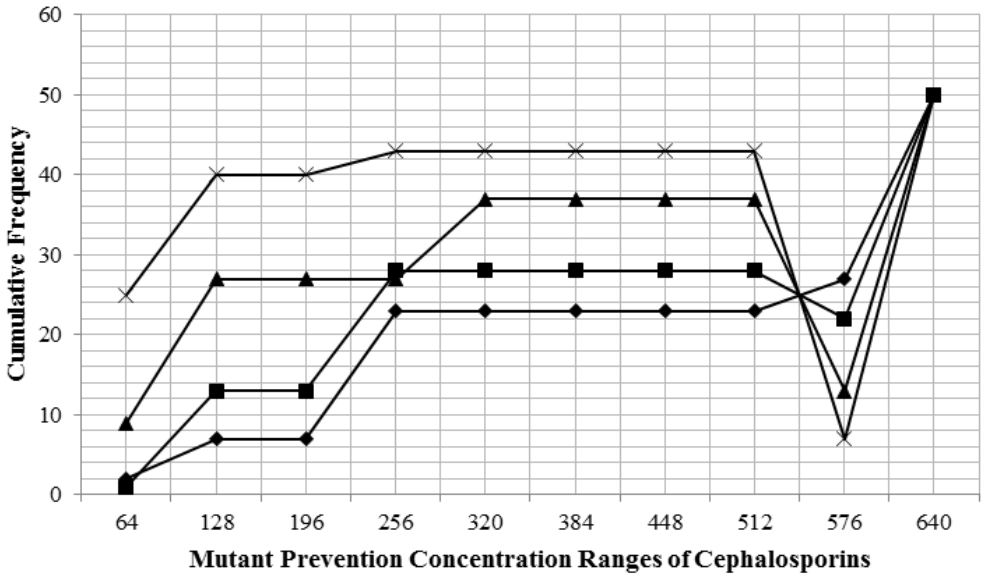

Figure 2: Cumulative Frequency Curves of cephalosporin MPCs for isolates of *Escherichia coli* (◆ Cephalexin, ■ Cefuroxime, ▲ Ceftazidime, X Cefepime).

The MPC_{50}/MIC_{50} and MPC_{90}/MIC_{90} ratio of cephalexin, cefuroxime, ceftazidime and cefepime for *E. coli* isolates were the same at 37°C and 41°C. The MPC_{50}/MIC_{50} ratios decreases in the order: cephalexin < cefuroxime < ceftazidime < cefepime as shown in Table 3. A lower value of the MPC/MIC ratio indicates a better ability to prevent the emergence of resistance mutants (Ozawa & Asai, 2013).

Table 3: Mutant Prevention Concentration/Minimum Inhibitory Concentration ratio of some cephalosporins for 50% and 90% of fecal *Escherichia coli* isolates at 37°C and 41°C

Antibiotics	MPC/MIC Ranges	Mutant Prevention Concentration/ Minimum Inhibitory Concentration	
		MPC_{50}/MIC_{50}	MPC_{90}/MIC_{90}
Cephalexin	2.0-64.0	6.2 (≤ 8.0)	6.9 (≤ 8.0)
Cefuroxime	1.0-32.0	3.3 (≤ 4.0)	7.3 (≤ 8.0)
Ceftazidime	1.0-32.0	2.2 (≤ 4.0)	12.2 (≤ 16.0)
Cefepime	1.0-64.0	1.4 (≤ 2.0)	20.6 (≤ 32.0)

MPC_{50}/MIC_{50} = **Mutant Prevention Concentration/Minimum Inhibitory Concentration ratio for 50% of isolates;** MPC_{90}/MIC_{90} = **Mutant Prevention Concentration/Minimum Inhibitory Concentration ratio for 90% of isolates.**

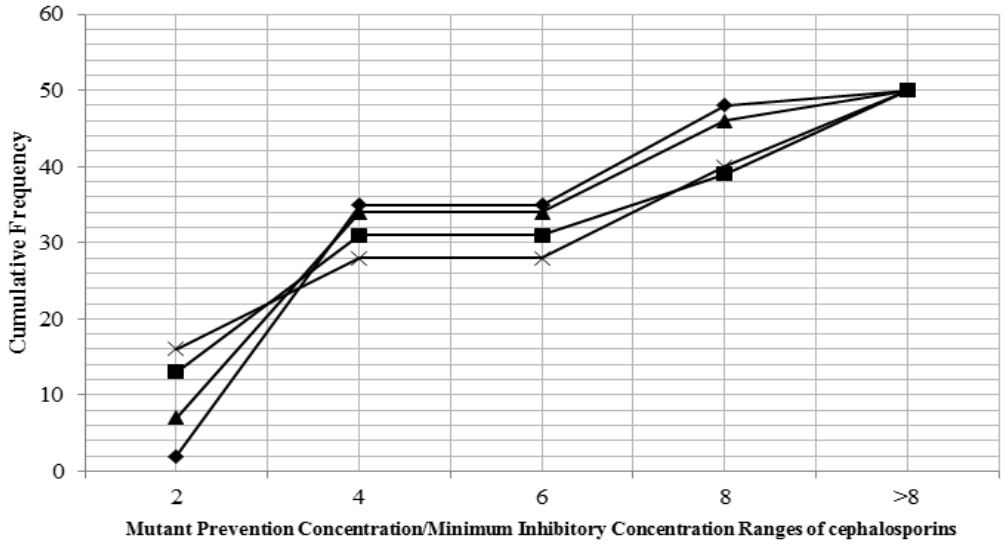

Figure 3: Cumulative Frequency Curves of cephalosporin MPC/MIC ratios for isolates of *Escherichia coli* (◆ Cephalexin, ■ Cefuroxime, ▲ Ceftazidime, X Cefepime).

Mutant Recovery

The mutant recovery (in percentage) at MPC_{50} (MR_{50}) and at MPC_{90} (MR_{90}) of cephalexin, cefuroxime, ceftazidime and cefepime for *E. coli* at 37°C and 41°C is as shown in Table 4.

Table 4: Mutant recovery (%) at MPC_{50} and MPC_{90} of some cephalosporins for fecal *Escherichia coli* isolates at 37°C and 41°C

Antibiotics	Mutant recovery (%) at MPC_{50} (MR_{50})		Mutant recovery (%) at MPC_{90} (MR_{90})	
	37°C	41°C	37°C	41°C
Cephalexin	$8.5 \times 10^7 \pm 1.00$	$8.3 \times 10^7 \pm 3.00$	$8.4 \times 10^7 \pm 2.00$	$8.3 \times 10^7 \pm 3.00$
Cefuroxime	$6.4 \times 10^7 \pm 2.00$	$6.7 \times 10^7 \pm 1.00$	$6.1 \times 10^7 \pm 3.00$	$7.1 \times 10^7 \pm 2.00$
Ceftazidime	$7.3 \times 10^7 \pm 1.00$	$8.5 \times 10^7 \pm 1.00$	$6.6 \times 10^7 \pm 1.00$	$7.1 \times 10^7 \pm 1.00$
Cefepime	$6.8 \times 10^7 \pm 1.00$	$8.6 \times 10^7 \pm 1.00$	$6.2 \times 10^7 \pm 1.00$	$7.7 \times 10^7 \pm 3.00$

MR_{50} = **Mutant recovery at MPC_{50};** MR_{90} = **Mutant recovery at MPC_{90}.**

Statistical Analyses

The MR_{50} of cephalexin, cefuroxime, ceftazidime and cefepime for *E. coli* isolates at both 37°C and 41°C were compared; MR_{90} of cephalexin, cefuroxime, ceftazidime and cefepime for *E. coli* isolates at both 37°C and 41°C were also compared as shown in Table 5. Generally differences between the mutant recovery at 37°C and 41°C were insignificant for all cephalosporins tested. However, differences in mutant recovery between the cephalosporins at 37°C were significant.

Discussion

Resistance in *E. coli* isolates to cephalosporins is increasingly reported (Akins et al., 2002; Forward et al., 2004; Johnson et al., 2007; Thokar et al., 2010; Drawz & Bonomo, 2010). Anti-mutant dosing has been advocated as a way to limit antimicrobial resistance (Liang et al., 2011).

Table 5: Statistical analyses of mutant recovery at MPC_{50} and MPC_{90} of some cephalosporins for fecal *Escherichia coli* isolates at 37°C and 41°C

Statistics	P value	Remarks (at P = 0.05)
MR_{50} Cephalexin (37°C vs. 41°C)	0.5918	Insignificant
MR_{50} Cefuroxime (37°C vs. 41°C)	0.6335	Insignificant
MR_{50} Ceftazidime (37°C vs. 41°C)	0.2318	Insignificant
MR_{50} Cefepime (37°C vs. 41°C)	0.0862	Insignificant
MR_{90} Cephalexin (37°C vs. 41°C)	0.8075	Insignificant
MR_{90} Cefuroxime (37°C vs. 41°C)	0.3196	Insignificant
MR_{90} Ceftazidime (37°C vs. 41°C)	0.4975	Insignificant
MR_{90} Cefepime (37°C vs. 41°C)	0.1548	Insignificant
MR_{50} at 37°C (for all the drugs)	0.0430	Significant
MR_{50} at 41°C (for all the drugs)	0.0973	Insignificant
MR_{90} at 37°C (for all the drugs)	0.1616	Insignificant
MR_{90} at 41°C (for all the drugs)	0.2633	Insignificant

MR_{50} = **Mutant Recovery at MPC_{50};** MR_{90} = **Mutant Recovery at MPC_{90}.**

This employs a therapeutic drug concentration, called 'mutant prevention concentration' (MPC) (Dong et al., 1999), which prevents the growth of the least susceptible single-step mutant present in a large bacterial population. MPC can be employed to compare the power of different antimicrobial agents to prevent emergence of antimicrobial resistance (Drlica, 2001). This study evaluated the MPCs of cephalexin, cefuroxime, ceftazidime and cefepime against fecal *E. coli* isolated in Keffi at 37°C and 41°C.

The MICs of cephalexin, cefuroxime, ceftazidime and cefepime obtained from this study are above the MIC breakpoint of 8.0 µg/ml (CLSI, 2012) suggesting that the organisms are resistant to the antibiotics tested. This observation is in agreement with an earlier report in Nigeria (Chigor et al., 2010). The observation that MPC_{50} and MPC_{90} for *E. coli* decreases with increasing generation of the cephalosporin suggest that the ability to develop resistance by mutation decreases with higher and newer generations of cephalosporins. The lack of difference in the MPC_{50} and MPC_{90} values of cephalosporins obtained when evaluated at 37°C or 41°C for each drug indicates that a rise in body temperature that usually accompany fever may not affect the concentration at which the development of resistance mutants is prevented.

The decrease in the MPC/MIC ratio with increasing generation of the cephalosporins suggests that higher generations of cephalosporins are less prone to developing resistance mutation (Ozawa & Asai, 2013). The higher mutant recovery observed at 41°C in this study suggest that temperature affects the extent of recovery of resistance mutant in *E. coli* following its exposure to cephalosporins. The relatively higher mutant recovery at 41°C suggests that the selection and enrichment of resistance mutants can be encouraged by a rise in temperature as usually observed during fever. This observation agrees with earlier reports with *E. coli* on the enhanced recovery of resistance mutants against aminoglycosides at higher temperature (Ngwai et al., 2013); and

fluoroquinolones under different temperatures (27°C or 37°C) and oxygen tension (aerobic or anaerobic atmosphere) (Linde & Lehn, 2004).

Conclusion

In 50% of the fecal *E. coli* isolated in Keffi, 6X the MIC of the cephalexin, 3X the MIC of cefuroxime, 2X the MIC of ceftazidime and 1X the MIC of cefepime are required to prevent development of single-step resistance mutants. *Escherichia coli* develop resistance mutation less likely with higher than lower generations of cephalosporin antibiotics. In addition, temperature has no effect on the prevention of selection and enrichment of resistance mutants against the cephalosporins tested, but enhances the extent of recovery of mutants providing a basis for the common practice of administrating high dose of antimicrobial agent at high body temperature during therapy of bacterial disease.

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References

Akins RL, Haase KK, Morris AJ (2002). Comparison of various fluoroquinolones (FQs) and four other antibiotics by mutant prevention concentration (MPC) against multi-drug resistant gram-negatives utilizing kill curves based on MPC-derived doses. In: Program and

Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA: American Society for Microbiology, Washington, DC, USA, A-1211. p10.

Cheesbrough M (2000). District Laboratory practice in Tropical Countries, Part 2. United Kingdom: Cambridge University Press. pp63-70.

Chigor VN, Umoh VJ, Smith SI, Igbinosa EO, Okoh AI (2010). Multidrug Resistance and Plasmid Patterns of *Escherichia coli* O157 and Other *E. coli* Isolated from Diarrhoeal Stools and Surface Waters from Some Selected Sources in Zaria, Nigeria. *Int J Environ Res Public Health* 7(10): 3831-3841.

CLSI (2012). *Methods for Dilution Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard- Ninth Edition (M07-A9)*. Wayne, PA: Clinical and Laboratory Standards Institute.

Credito K, Kosowska-Shick K, McGhee P, Pankuch GA, Appelbaum PC (2010). Comparative study of the mutant prevention concentrations of Moxifloxacin, Levofloxacin, and Gemifloxacin against pneumococci. *Antimicrob Agents Chemother* 54: 673-677.

Dong Y, Zhao X, Domagala J, Drlica K (1999). Effect of fluoroquinolone concentration on selection of resistant mutants of *Mycobacterium bovis* BCG and *Staphylococcus aureus*. *Antimicrob Agents Chemother* 43:1756-1758.

Drawz SM, Bonomo RA (2010). Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev* 23:160-201.

Drlica K, Zhao X (2007). Mutant Selection Window Hypothesis updated. *Clin Infect Dis* 44: 681-688.

Drlica K (2001). A strategy for fighting antibiotic resistance. *ASM News* 67:27-33.

Forward KR, Matheson KM, Hiltz M, Musgrave H, Poppe C (2004). Recovery of cephalosporin-resistant Escherichia coli and Salmonella from pork, beef and chicken marketed in Nova Scotia. *Can J Infect Dis Med Microbiol* 15(4):226-230.

Johnson JR, Sannes MR, Croy C, Johnston B, Clabots C, Kuskowski MA, Bender J, Smith KE, Winokur PL, Belongia EA (2007). Antimicrobial Drug-Resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002–2004. *Emerg Infect Dis* 13(6). http://wwwnc.cdc.gov/eid/article/13/6/06-1576.htm. [Retrieved: *15/05/2013*].

Liang B, Bai N, Cai Y, Wang R, Drlica K, Zhao X (2011). Mutant Prevention Concentration-Based Pharmacokinetic/Pharmacodynamic Indices as Dosing Targets for Suppressing the Enrichment of Levofloxacin-Resistant Subpopulations of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 55(5):2409-2412.

Linde H-J, Lehn N (2004). Mutant Prevention Concentration of Nalidixic Acid, clinafloxacin, Levofloxacin or Trovafloxacin for *Escherichia coli* under different growth condition. *Antimicrob Chemother* 53:252-257.

Ngwai YB, Shekwodza CA, Nkene IH, Owuna GE, Envuladu EY (2013). Mutant Prevention Concentrations of some Aminoglycoside Antibiotics for fecal isolates of Escherichia coli under different growth temperatures. *J Nat Sci Res* 3(7):147-154.

Ozawa M, Asai T (2013). Relationships between mutant prevention concentrations and mutation frequencies against enrofloxacin for avian pathogenic *Escherichia coli* isolates. *J Vet Med Sci* 75(6):709-713.

Randall LP, Cooles SW, Sayers AR, Woodward MJ (2001). Cyclohexane resistance in *Salmonella* of different serovars is associated with increased resistance to multiple antibiotics, disinfectants and dyes. *J Med Microbiol* 50:1-6.

Roberts JA, Kruger P, Paterson DL, Lipman J (2008). Antibiotic resistance - what's dosing got to do with it? *Crit Care Med* 36:2433-2440.

Salyers AA, Gupta A, Wang Y (2004). "Human intestinal bacteria as reservoirs for antibiotic resistance genes". *Trends Microbiol* 12(9):412-416.

Thokar MA, Fomda BA, Maroof P,Ahmed K, Bashir D, Bashir G (2010). Proliferation of extended spectrum beta-Lactamase (ESBL) producing gram negative bacteria, diagnostic inputs and impact on selection of antimicrobial therapy. *Physicians Acad* 4:25-31.

Todar K (2007). "Pathogenic *Escherichia coli*." Online Textbook of Bacteriology. University of Wisconsin-Madison. Department of Bacteriology. http://www.textbookofbacterioloogy.net/e.coli.html. [Retrieved: 03-05-2013].