Original Article

Escherichia coli from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams

Samuel Kariuki,[1] Gunturu Revathi,[2] John Corkill,[3] John Kiiru,[1] Joyce Mwituria,[1] Nazir Mirza,[4] C. Anthony Hart.[3]

[1]Centre for Microbiology Research, Kenya Medical Research Institute, PO Box 43640, Nairobi, Kenya, [2]Department of Pathology, Aga Khan University Hospital, P.O. Box 30270, Nairobi, Kenya, [3]Department of Medical Microbiology and Genito-Urinary Medicine, University of Liverpool, Liverpool, L69 3GA UK, [4]The Nairobi Hospital, PO Box 30026, Nairobi, Kenya.

Abstract

Background: Uropathogenic *Escherichia coli* are increasingly becoming resistant to flouroquinolones and to other commonly available antimicrobials. We sought to investigate the genetic basis for fluoroquinolone and extended spectrum beta-lactam (ESBL) resistance in 17 fluoroquinolone-resistant (MIC of levofloxacin and ciprofloxacin >32 µg/ml) *E. coli* isolated from patients with urinary tract infections (UTIs).

Methods: We applied PCR and Pulsed Field Gel Electrophoresis (PFGE) to characterize resistance genes and to determine clonal relatedness of strains, respectively.

Results: Twelve of the 17 *E. coli* were resistant to multiple drugs, including ampicillin, co-amoxyclav, cefotaxime, ceftriaxone, ceftazidime and gentamicin and nalidixic acid and produced plasmid-mediated CTX-M-15 type ESBLs and CMY-2 AmpC type enzymes. The other 5 *E. coli* that were non-ESBL-producing were multiply resistant to ampicillin, nitrofurantoin, cefoxitin, nalidixic acid. Resistance to fluoroquinolones resulted from a combination of the presence of qnrA, qnrB, ciprofloxacin acetylating enzyme designated aac(6')-1b-cr, and mutations in the two amino acid substitutions; 83 Serine (TCG) to Leucine (TTG) and 87 Aspartic acid (GAC) to Asparagine (AAC).

Conclusion: Antibiogram patterns and PFGE of *E. coli* showed that these were community acquired UTI caused by pockets of clonally-related and some discreet strain types. Plasmid-mediated CTX-M-15 beta-lactamases and CMY-2 AmpC enzymes and fluoroquinolone resistant *E. coli* are becoming increasingly prevalent in hospitals in Kenya, posing a major challenge in the management of UTIs.

Key Words: Fluoroquinolone-resistant, ESBL-producing MDR uropathogenic *E. coli*, Kenya.

J Infect Developing Countries 2007; 1(3):257-262.

Received 18 September 2007 - Accepted 22 October 2007.

Introduction

Urinary tract infections (UTIs) including cystitis and pyelonephritis are common both in community and hospital settings [1-3] with *Escherichia coli* being the predominant pathogen [4]. However, other organisms gain a greater foothold in patients with complicated UTI [5]. Although uncomplicated UTIs caused by *E. coli* and other Enterobacteriaceae should be treated empirically with co-trimoxazole [6,7] the rates of community-acquired strains with decreasing susceptibility to first-line agents such as ampicillin, nitrofurantoin, and co-trimoxazole have been on the increase. More recently resistance to fluoroquinolones such as ciprofloxacin and levofloxacin has been on the increase [1,8-10]. It is therefore important that antibiotic resistance profiles at the local level must be known in order to support an empiric approach to the management of these infections. We present data on antimicrobial susceptibility and the genetic basis of resistance in multidrug resistant *E. coli* isolates from community-acquired UTIs in patients attending two hospitals in Nairobi, Kenya.

Materials and Methods

Patients

Patients with symptoms of urinary tract infection (UTI) were studied between June 2004 and August 2005 at The Aga Khan University Hospital, Kenya. During this period the respective laboratories isolated a total of 64 multidrug-resistant (MDR) *E. coli* isolates (resistant to at least ampicillin and cotrimoxazole) representing 36% of all *E. coli* isolations from routine urine

cultures. A total of 17 of the MDR *E. coli* were initially resistant to ciprofloxacin and levofloxacin by routine disk diffusion methods and these isolates were selected for further study. They were isolated from 15 adults and two children (a 6-month-old baby boy and 12-year-old girl) with UTIs. Eleven of the adults and the 2 children were treated as outpatients; one adult each was admitted to the surgical and medical wards, respectively. All identifying clinical information was removed from the clinic data base and permission to further test the *E. coli* strains after isolation during routine clinical care was obtained from the KEMRI/National Ethical Review Board.

Antibiotic susceptibility testing

Initially all isolates were tested for susceptibility to ampicillin, co-amoxyclav, ceftazidime, cefotaxime, ceftriaxone, co-trimoxazole, gentamicin, cefoxitin, cefepime, nalidixic acid, ciprofloxacin, imipenem, meropenem, tetracycline and nitrofurantoin (Oxoid Ltd., Basingstoke, United Kingdom) using E-Test MIC strips (E-test; AB BioDisk, Solna, Sweden) on Mueller-Hinton agar (Oxoid) as described by the Clinical Laboratory Standards Institute [11]. In addition, testing of susceptibility to cefotaxime, cefepime and to ceftazidime alone (E-test; AB BioDisk) and in combination with clavulanic acid (E-test for ESBLs; AB BioDisk) was performed on donors and *E. coli* K-12 transconjugants. Screening to detect phenotypic production of AmpC β-lactamases was done by direct susceptibility testing with a cefoxitin 30-µg disk and the three-dimensional enzyme extract test [12]. *Escherichia coli* ATCC® 25922 and ATCC® 35218 were included in each test to control for growth and potency in tests for MICs and for β-lactam/β-lactamase inhibitor combinations, respectively.

Pulsed-field gel electrophoresis

Initially *E. coli* cultures were grown overnight in Nutrient broth (Oxoid), and the bacterial cells were then embedded in agarose plugs and chromosomal DNA obtained by digestion with Lysozyme (25 mg/mL) (Sigma, Poole, United Kingdom). Chromosomal DNA in agarose plugs was digested using 35 U of XbaI and 25 U of SpeI (Life Technologies, Paisley, United Kingdom) using a previously developed protocol [13]. PFGE of agarose plug inserts was then performed on a CHEF-DR II system (Bio-Rad Laboratories, Richmond, Calif.) on a horizontal 1% agarose gel for 22 h at 120 V, with a pulse time of 1 to 40 s at 14℃. A lambda DNA digest consisting of a ladder (ca. 22 fragments) of increasing size from 48 kb to approximately 1,000 kb was included as a DNA size standard. The gel was stained with ethidium bromide and photographed on a UV transilluminator (UVP Inc., San Gabriel, Calif.). The restriction endonuclease (RE) digest patterns were compared, and their similarities were scored by the method of Tenover *et al.* (14). By these criteria isolates that gave PFGE banding patterns that were indistinguishable were assumed to be from a single outbreak strain. Isolates that gave banding patterns showing differences in fewer than four bands were assumed to be closely related, as they may represent isolates differing by a single genetic event. In addition, isolates with differences in four to six bands may also be part of an outbreak. However, isolates showing a difference in more than seven bands of their banding patterns may represent more than three genetic events, in which case they were considered epidemiologically unrelated.

In-vitro conjugation and plasmid analysis

Each of the 17 MDR *E. coli* were conjugated with an azide resistant strain of *E. coli* J53, according to a previously described procedure [12] and plated on Mueller-Hinton agar containing ampicillin (32 µg/ml) and Sodium azide (200 µg/ml). The putative transconjugants were tested for susceptibility to all 15 antimicrobial agents by a disk-diffusion method to identify transferable resistance. Plasmid DNA extraction from both donor and transconjugants was performed using a Plasmid Mini Prep Kit (Qiagen Ltd., West Sussex, United Kingdom) according to manufacturer's instructions.

Extraction of DNA template for PCR

Cells from 1.5 ml of the overnight culture were harvested by centrifugation at 13,000 × g for 5 min. After the supernatant was decanted, the pellet was resuspended in 5% Chelex-100 resin slurry (Bio-Rad) in injection grade water followed by boiling for 10 min. The supernatant, 2 µl (1/250 volume) of the total sample, was used as the source of template DNA for amplification.

PCR for CTX-M genes

PCR amplification of the entire coding sequence of the blaCTX-M gene (ca. 1-kb amplicon) was done by the method described by Gniadowski *et al*. [15]. using primers P1C (5′-TCG TCT CTT CCA GA-3′) and P2D (5′-CAG CGC TTT TGC CGT CTA AG-3′).

PCR for fluoroquinolone resistance and AmpC gene

PCR of the quinolone resistance determining region (QRDR) was performed with the four primer pairs as described previously [13]. In addition we applied qnr gene primers, QP1 (5′-ATAAAGTTTTTCAGCAAGAGG; starting at the 12th qnr nucleotide) and QP2 (5′-ATCCAGATCGGCAAAGGTTA), to detect qnrA gene on plasmids (length, 657 bp). For qnrB, primers FQ1 (5'-ATGACGCCATTACTGTATAA) and FQ2 (5'GATCGCAATGTGTGAAGTTT) were used [16]. Two sets of primers, aac(6')-1b-cr 1 [aac(6')-1b-cr1 (5'ATATGCGGATCCAATGAGCAA CGCAAAAACAAAGTTAG3') aac(6')-1b-cr1 (5'ATATGCGAATTCTTAGGCATCACTGCGTGTT CGCTC3')] and aac(6')-1b-cr 2 [aac(6')-1b-cr2 (5'TTGCAATGCTGAATGGAGAG3'), aac(6')-1b-cr2 (5'CGTTTGGATCTTGGTGACCT3')] were used to detect ciprofloxacin acetylating enzyme designated aac(6')-1b-cr [(confers low level ciprofloxacin resistance (MIC=0.25 µg/ml)] and located on a mobile transposon. Amplification conditions consisted of 30 cycles of 94℃ for 30 s, 55℃ for 30 s, and 72℃ for 30 s, with a final extension step of 72℃ for 10 min. The primers used for the multiplex PCR amplification of AmpC gene are listed in the Table. For all PCRs, amplification conditions consisted of initial denaturation at 94℃ for 3 min, followed by 25 cycles at 94℃ for 30s, 64℃ for 30s, 72℃ for 1 min, and a final extension at 72℃ for 7 min. PCR products were resolved by electrophoresis on 1.2% gels at 120 V run for 1 h.

Sequence determination

Sequence determination was performed using the PCR primers for both strands of the amplicons with a dideoxy-chain termination method using an automated DNA sequencer ABI PRISM 377 (Perkin-Elmer, Warrington, United Kingdom) and was analyzed using commercial software (Lasergene; DNAStar Inc., Madison, Wis.).

Table 1. Primers used for PCR amplification and sequencing of the CMY gene

Primer	Primer sequence	GenBank Accession No.
MOXM-F	5′-GCTGCTGAAGGAGCACAGGAT-3′	D13304
MOXM-R	5′-CACATTGACATAGGTGTGGTGC-3′	
CITM-R	5′-TGGCCAGAACTGACAGGCAAA-3′	X78117
CITM-R	5′-TTT CTCCTGAACGTGGCTGGC-3′	
DHAM-F	5′-AACTTTCACAGGTGTGCTGGGT-3′	Y16410
DHAM-R	5′-CCGTACGCATACTGGCTTTGC-3′	
ACCM-F	5′-AACAGCCTCAGCAGCCGGTTA-3′	AJ133121
ACCM-R	5′-TTCGCCGCAATCATCCCTAGC-3′	
EBCM-F	5′-TCGGTAAAGCCGATG TTGCGG-3′	M37839
EBCM-R	5′-CTTCCACTGCGGCTGCCACTT-3′	
FOXM-F	5′-AACATGGGGTATCAGGGAGATG	X77455
FOXM-R	5′-CAAAGCGCGTAACCGGATTGG	

Results

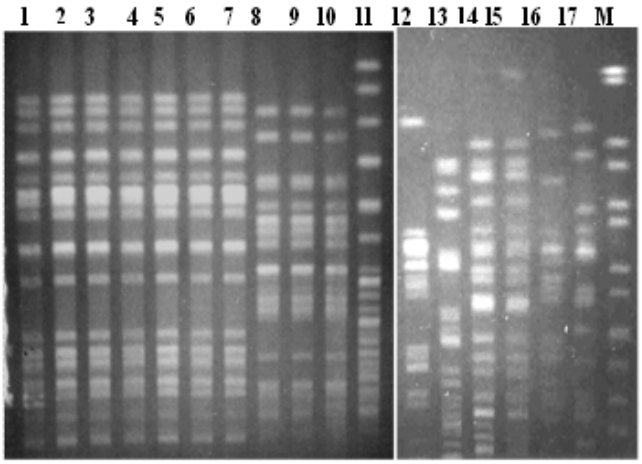
Twelve out of the 17 *E. coli* isolates were resistant to ampicillin, ceftazidime, cefotaxime, ceftriaxone, tetracycline, nalidixic acid (MICs >256 µg/ml), gentamicin (MIC 24-32 µg/ml), ciprofloxacin and levofloxacin (MICs >32 µg/ml). They showed intermediate resistance to co-amoxyclav (MIC= 8-16 µg/ml) and cefoxitin (MIC 12-16 µg/ml) but were fully resistant to nitrofurantoin (MIC >128 µg/ml). Using ESBL E-test strips, these 12 *E. coli* also expressed cefotaximase and cefepimase activities, with correspondingly high MICs of the respective cephalosporins (MIC > 256 µg/ml). The 5 other *E. coli* that were non-ESBL producers were fully sensitive to gentamicin, cefotaxime, ceftriaxone, ceftazidime and cefepime, and one isolate to co-trimoxazole, but they were resistant to ampicillin, co-amoxiclav, nalidixic acid, ciprofloxacin, levofloxacin, nitrofurantoin and cefoxitin. Imipenem and meropenem were the only antimicrobials to which each of the 17 MDR *E. coli* isolates were fully susceptible.

PFGE patterns of the *E. coli* isolates are shown in the Figure. Using either XbaI or SpeI-digested chromosomal DNA, there were three PFGE patterns for *E. coli* from Aga Khan Hospital. Pattern 1 contained 7 *E. coli* isolates, pattern 2 comprised 3 isolates and pattern 3 had only one isolate. Isolates within patterns 1 and 2 produced indistinguishable PFGE digest patterns but there

were 3-5 band differences between them. These isolates were from 3 different outpatient clinics and were obtained over a one-year period, and hence were likely to represent distinct pockets of community-acquired clonally-related strain types. In contrast all 6 isolates from Nairobi Hospital produced different PFGE digest patterns indicating that these were isolates from distinct and unrelated sources of infection in the respective communities.

All the 12 ESBL-producing *E. coli* contained a 42 kb plasmid in addition to two smaller plasmids of 4 and 2.5 kb. The non-ESBL producers all contained a 3 kb plasmid. *In vitro* conjugation tests showed that the 42 kb plasmid co-transferred resistance to ampicillin, cefotaxime, ceftriaxone, ceftazidime, cefepime, co-trimoxazole and tetracycline.

Figure 1. PFGE patterns of 11 XbaI-digested cefotaxime and ciprofloxacin resistant *E. coli* from cases of urinary tract infections attending a hospital in Nairobi. Lanes 1-11 are PFGE patterns of *E. coli* from Aga Khan Hospital, while lanes 12-17 shows PFGE patterns of E. coli from Nairobi Hospital. Lane M, 50 kb molecular size standard; Lanes 1 to 7, *E. coli* from main PFGE pattern 1 (all from outpatients); Lanes 9 to11, *E. coli* from PFGE pattern 2 (one from outpatient, one from surgical ward and 1 from medical ward); Lanes 12-17 *E. coli* from Nairobi Hospital (from outpatients) showing 5 distinct PFGE patterns.



Each of the 17 MDR *E. coli* isolates contained two amino acid substitutions in the QRDR, i.e. 83 Serine (TCG) to Leucine (TTG) and 87 Aspartic acid (GAC) to Asparagine (AAC), and two silent mutations in amino acids 156 Proline (CCC to CCG) and 182 Iso-leucine (ATC to ATT). In addition, all the MDR *E. coli* contained the aac(6')-1b-cr2 gene while 6/17 isolates also carried the qnrA and qnrB genes. The 5 cefoxitin resistant non-ESBL producing isolates were also positive for the AmpC gene, with amplicons of 462 bp, indicating that the genes probably arose from Citrobacter freundii. These isolates all carried a 3kb plasmid which was not transferred to *E. coli* J53 *in vitro*. The AmpC sequence showed high identity to CMY-2 but to none of the other AmpC genes tested. These 5 *E. coli* isolates were from patients seen at 2 separate outpatient clinics and one surgical ward at the Aga Khan Hospital. They are likely to be clonal as their XbaI and SpeI PFGE patterns were indistinguishable. PCR amplification of the entire coding sequence of the blaCTX-M gene revealed complete sequence identity for each of the 12 cefotaxime-resistant *E. coli* as well as from their *E. coli* J53 transconjugants with CTX-M-15.

Discussion

Worldwide *E. coli* is the major cause of UTIs and accounts for 75% to 90% of UTI isolates [2, 17]. The increasing prevalence of infections caused by antibiotic-resistant bacteria makes the empirical treatment of UTIs difficult and outcome unpredictable [6]. In the current study we examined 64 MDR *E. coli* – which represent 36% of all UTI isolates from two hospitals in Nairobi. A total of 17 *E. coli* were also resistant to fluoroquinolones and 12 of these were also resistant to each of the four extended spectrum beta-lactams, cefotaxime, ceftriaxone, ceftazidime and cefepime that form the mainstay of reserve antibiotics for treatment of severe infections in these hospitals. In Kenyan private clinics and hospitals, ciprofloxacin is the most frequently prescribed fluoroquinolone for UTIs because of its availability in oral and intravenous formulations. In poor-resource settings where the availability of alternative effective antibiotics is limited, serious problems are anticipated in the treatment of multidrug- and fluoroquinolone-resistant *E. coli*.

Many studies worldwide have also reported a sharp increase in ciprofloxacin resistant *E. coli* isolates from UTIs. For example, in China, from 1998 to 2002, the prevalence of ciprofloxacin resistance has increased steadily from 46.6% to 59.4% [6], and in Bangladesh the prevalence was 26% [8]. Although some studies have identified plasmid-mediated fluoroquinolone resistance in clinical bacterial isolates that showed reduced

susceptibility to fluoroquinolones [12,16] it appears that full resistance to fluoroquinolones (MICs > 32 ug/ml) in our *E. coli* isolates was due to point mutations in the QRDR, namely 83 Serine (TCG) to Leucine (TTG) and 87 Aspartic acid (GAC) to Asparagine (AAC), and qnrA, qnrB and aac(6')-1b-cr2 genes. However, we cannot exclude the possibility that the presence of the qnrS gene might have also contributed to the mutations in the QRDR. Each of the ESBL producing *E. coli* contained a plasmid mediated cefotaxime-hydrolysing CTX-M-15 enzyme encoded on a 42 kb plasmid which was also associated with transferable resistance to ampicillin, cefotaxime, ceftriaxone, ceftazidime, cefepime, co-trimoxazole and tetracycline. To our knowledge this is the first report of CTX-M-15 enzyme to be found in *E. coli* isolates from the region. Previously we identified a novel CTX-M-12 enzyme in a nosocomial isolate of Klebsiella pneumoniae from a local hospital [18]. Although we were unable to categorically determine prior usage of fluoroquinolones by individual patients, these antibiotics are the current drug of choice for treatment of UTIs and it is plausible that patients who had previous episodes of UTIs may already have been exposed to these antibiotics. Other studies in USA [19] and Tunisia [20] have also identified urinary *E. coli* that produced the plasmid-mediated AmpC enzyme beta-lactamase CMY-2 and CTX-M15 ESBLs, and these were resistant to ceftazidime, ceftriaxone, and cefepime but sensitive to meropenem. In Taiwan, *E. coli* isolates recovered from food animal feces, retail ground meats, and *urinary E. coli* isolates from outpatients were shown to carry a CMY-2 -lactamase [21].

The PFGE patterns of *E. coli* isolates from Aga Khan Hospital indicate that UTI infections in the outpatient group were likely to be from pockets of a clonally-related community-acquired strain while those that caused UTIs at the Nairobi Hospital may have originated from different sources. As these isolates were collected over a period of one year and were mainly from outpatients, it is plausible that the UTIs may have several sources of infection in the community as even isolates from inpatients showed distinct PFGE patterns. Previous studies in Canada [22] and USA [23] have also described the occurrence of several *E. coli* clonal groups in a community suggesting that a proportion of community-acquired UTIs may be caused by *E. coli* disseminated from one or more point sources.

In conclusion, antimicrobial resistance patterns and PFGE showed that these were community acquired *E. coli* UTIs caused by pockets of both clonally related and some discreet strains. These findings also show that plasmid-mediated CTX-M-15 and CMY-2 AmpC -lactamase, and fluoroquinolone resistance among urinary *E. coli* are prevalent, posing a major challenge in the management of community-acquired UTIs.

Acknowledgement

We thank the Director, Kenya Medical Research Institute, for permission to publish this work. We duly acknowledge Ms Jane Muyodi and Agnes Munyalo for technical assistance in the laboratory. Funding source: SK was supported by a Wellcome Trust Training Fellowship.

References

1. Karlowsky JA, Hoban DJ, Decorby MR, Laing NM, Zhanel GG (2006) Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from outpatients are frequently multidrug resistant: results from the North American Urinary Tract Infection Collaborative Alliance-Quinolone Resistance study. Antimicrob Agents Chemother 50:2251-4.
2. Nicolle LE (2001) Urinary tract infection: traditional pharmacologic therapies. Am J Med 113(Suppl. 1A):35–44.
3. Nicolle LE (2005) Catheter-related urinary tract infection. Drugs Aging 22:627-39.
4. Gupta K, Sahm DF, Mayfield D, Stamm WE (2001) Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. Clin Infect Dis 33:89–94.
5. Mathai D, Jones RN, Pfaller MA; SENTRY Participant Group North America (2001) Epidemiology and frequency of resistance among pathogens causing urinary tract infections in 1,510 hospitalized patients: a report from the SENTRY Antimicrobial Surveillance Program (North America). Diagn Microbiol Infect Dis 40:129–36.
6. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE (1999) Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Clin Infect Dis 29:745–58.
7. Zhanel GG, Karlowsky JA, Harding GKM, Carrie A, Mazzulli T, Low DE; The Canadian Urinary Isolate Study Group (2000) A Canadian national surveillance study of urinary tract isolates from outpatients: comparison of the activities of trimethoprim–sulfamethoxazole, ampicillin, mecillinam, nitrofurantoin, and ciprofloxacin. Antimicrob Agents Chemother 44:1089–92.
8. Gupta K, Hooton TM, Stamm WE (2001) Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann Intern Med 135:41–50.
9. Hooton TM (2003) Fluoroquinolones and resistance in the treatment of uncomplicated urinary tract infection. Int J Antimicrob Agents 2: 65–72.

10. Karlowsky JA, Thornsberry C, Jones ME, Sahm DF (2003) Susceptibility of antimicrobial-resistant urinary *Escherichia coli* isolates to fluoroquinolones and nitrofurantoin. Clin Infect Dis 36:183–7.
11. Clinical Laboratory Standards Institute (2005) Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement M100-S15, Vol. 25 No. 1. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. Corkill JE, Anson JJ, Hart CA (2005) High prevalence of the plasmid-mediated quinolone resistance determinant qnrA in multidrug-resistant Enterobacteriaceae from blood cultures in Liverpool, UK. J Antimicrob Chemother 56:1115-7.
13. Kariuki S, Revathi G, Muyodi J, Mwituria J, Munyalo A, Mirza S, Hart CA (2004) Characterization of multidrug-resistant typhoid outbreaks in Kenya. J Clin Microbiol 42:1477-82.
14. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233–9.
15. Gniadkowski M, Schneider I, Palucha A, Jungwirth R, Mikiewicz B, Bauernfeind A (1998) Cefotaxime-resistant Enterobacteriaceae isolates from a hospital in Warsaw, Poland: identification of a new CTX-M-3 cefotaxime-hydrolyzing β-lactamase that is closely related to the CTX-M-1/MENN-1 enzyme. Antimicrob Agents Chemother 42:827–32.
16. Jacoby GA, Walsh KE, Mills DM, Walker VJ, Oh H, Robicsek A, Hooper DC (2006) qnrB, another plasmid-mediated gene for quinolone resistance. Antimicrob Agents Chemother 50:1178-82.
17. Huang ES, Stafford RS (2002) National patterns in the treatment of urinary tract infections in women by ambulatory care physicians. Arch Intern Med 162:41–7.
18. Kariuki S, Corkill JE, Revathi G, Musoke R, Hart CA (2001) Molecular characterization of a novel plasmid-encoded cefotaximase (CTX-M-12) found in clinical Klebsiella pneumoniae isolates from Kenya. Antimicrob. Agents Chemother. 45:2141-3.
19. Odeh R, Kelkar S, Hujer AM, Bonomo RA, Schreckenberger PC, Quinn JP (2002) Broad resistance due to plasmid-mediated AmpC beta-lactamases in clinical isolates of *Escherichia coli.* Clin Infect Dis 35:140-5.
20. Bauernfeind A, Hohl P, Schneider I, Jungwirth R, Frei R (1998) *Escherichia coli* producing a cephamycinase (CMY-2) from a patient from the Libyan-Tunisian border region. Clin Microbiol Infect 4:168-70.
21. Yan JJ, Hong CY, Ko WC, Chen YJ, Tsai SH, Chuang CL, Wu JJ (2204) Dissemination of blaCMY-2 among *Escherichia coli* isolates from food animals, retail ground meats, and humans in southern Taiwan. Antimicrob Agents Chemother 48:1353-6.
22. Pitout JD, Gregson DB, Church DL, Elsayed S, Laupland KB (2004) Community-wide outbreaks of clonally related CTX-M-14 beta-lactamase-producing *Escherichia coli* strains in the Calgary health region. Clin Infect Dis 38:329-34.
23. Manges AR, Dietrich PS, Riley LW (2004) Multidrug-resistant *Escherichia coli* clonal groups causing community-acquired pyelonephritis. Clin Infect Dis 38:329-34.

Corresponding Author: Samuel Kariuki, Centre for Microbiology Research, Kenya Medical Research Institute, PO Box 43640, 00100 Nairobi, Kenya. Phone: 254.202. 718247. E-mail: skariuki@kemri.org

Conflict of Interests: The authors declare that they have no conflict of interests.