Current Research Journal of Biological Sciences 4(6): 696-701, 2012
ISSN: 2041-0778

Submitted: August 16, 2012 Accepted: September 24, 2012 Published: November 20, 2012

Antibiotic Resistance in *Escherichia coli* Isolated from Women Genitalia and Trend of Minimal Inhibiting Concentration in a Semi-Urban Population: Bangangté, West-Cameroon

[1, 2]Fotsing Kwetche Pierre René, [1, 2]Kouamouo Jonas, [1, 2]Mbaya Patrice, [1, 2]Yangoué Diane
[1, 2]Nsangou Adamou, [1, 2]Wona Jean Pierre, [1, 2]Simo Louokdom Josué,
[1, 2]Kourouma Kaba and [1, 2]Kaptué Lazare
[1]Institut des Sciences de la Santé de Bangangté, Université des Montagnes,
[2]Laboratory of Microbiology, Cliniques Universitaires des Montagnes, Bangangté-Cameroon (French)

Abstract: The present survey aimed at assessing drug availability, frequencies of resistant *E. coli* isolated from women genitalia and Minimal Inhibiting Concentrations (MIC) of common antibiotics provided by formal and informal networks in Bangangté. Susceptibility tests were performed by disc diffusion and the MIC by agar dilution techniques. The MIC tests were conducted for tetracycline, chloramphenicol and ampicillin, on two reference strains; *E. coli* and *S. aureus*. Associations between these parameters were also evaluated. Data analysis revealed that most available antibiotics include Thrimethoprim/sulfamethoxazol 97.05%, amoxicillin 94%, ampicillin 85.29%, ciprofloxacin 70% and gentamycin 29.41%. The resistance rates associated were estimated at 82, 64, 78, 52 and 18%, respectively. In addition, antibiotics provided by informal drug-selling network displayed higher MIC values. They may represent a key driving force facilitating selection of resistant bacterial strains. These findings are evidences that antimicrobial resistance is a crucial challenging issue to address in the setting, highlighting the necessity to undertake a larger-scale survey that will help mass-education in order to discourage the current trend of antimicrobial resistance and re-orient antibiotic-based chemotherapy.

Keywords: Antimicrobial, drug-selling, networks, resistance

INTRODUCTION

Microbial drug resistance is a crucial health issue that can seriously affect the economic and social standards of populations in both industrialized and developing countries across the world. Resistance phenotypes reported in bacteria can either be natural or acquired. Natural resistance refers to the inherent ability observed in all strains of a species to survive and grow in the presence of an anti-infectious agent while acquired resistance is the one observed in some strains of a species all members of which were previously susceptible to that agent. The impact of resistant microorganisms is obvious in hospitals and other healthcare facilities, when infections caused by drug-resistant microorganisms fail to respond to standard drug therapy. This results in a prolonged period of infectivity with the related morbidities and deaths, especially among immunocompromised patients (McGowan, 1983; Chow *et al*., 1991; Cuzon *et al*., 2011).

The frequency rates and resistance profiles of microorganisms may vary from one area to the other, depending upon such parameters as population purchasing power, standard lifestyle and level of education (Bisht *et al*., 2009). The World Health Organization (WHO, 2002) estimated that about 80% of antibiotics consumption takes place in the community for human use and that, at least half of this is based on inadequate indication, mostly viral infections, that normally require symptomatic rather than drug therapy. In industrialized countries, driving forces known to promote anti-microbial resistance basically include the use of antibiotics for growth promotion in animals (Zhang *et al*., 2006; Jacob *et al*., 2008; Bisht *et al*., 2009; Wagner *et al*., 2011; Espinal *et al*., 2011), pesticides in agriculture and frequent prescription of most recent antibiotics (last line) by physicians (Bisht *et al*., 2009). In resource-limited countries, factors like monotherapy, inadequate dosage associated with treatment interruption are most likely responsible for resistance phenotype growth among bacteria (Cohen, 1992; Gold and Moellering, 1996). In these areas, the limited number of antibiotics is increasingly inadequate for infectious disease management (Roger *et al*., 2003; Shetty *et al*., 2009; Andersson and Hughes, 2011). Therefore, if the economic and health impact of resistance is a major threat in industrialized nations, they are more severe in developing countries (Malonza *et al*., 1997; Fasehun, 1999; Edoh and Alomatu, 2008).

Corresponding Author: Fotsing Kwetche Pierre René, Institut des Sciences de la Santé de Bangangté, Université des Montagnes, P.O Box 208 Bangangté, Cameroon (French), Tel.: 23777018171/23799619786

Nowadays, the facilitated transfer of peoples and goods across borders that accompanies globalization enables rapid spread of resistance from one area to the other, regardless of the living standards (WHO, 2011). To maintain drug effectiveness for a longer period, the need for monitoring the use of anti-infectious agents is paramount in all settings (Newman *et al*., 2011). So far, limited data exist on this crucial public health issue in many rural and semi-urban areas, especially in developing nations, due to lack of resources required for research and education. Difficulties to treat bacterial infections in Bangangté (a semi-rural area situated in West Cameroon) prompted us to initiate this study which aimed at: assessing the resistance phenotypes of *E. coli* isolated from women genitalia, evaluating drug availability as indicator of usage tendency and determining the Minimal Inhibiting Concentrations (MIC) of three common antibiotics provided by formal and informal selling networks in order to appreciate their dose-based quality. Expected results will be used as database for mass education on the appropriate use of anti-infectious agents in semi-urban and rural communities.

MATERIALS AND METHODS

Setting, population and drug utilization: The present study was conducted from January to November 2011 in Bangangté, a semi-urban area in West Cameroon where basic income-generating activities are agriculture and animal husbandry.

Participants were submitted to a series of questions including the use of antimicrobial before reporting to the hospital, their drug providers (pharmacies, groceries, open ordinary market place), the types of drug, the prescribing authority (medical doctor, nurse). In a parallel manner, drug selling was investigated based on anonymous identification of drug-selling structures and the type of drugs each one could provide.

Specimen collection and culture: Biological specimens were collected from women patients who accepted to participate after ethical requirements were fulfilled. Prior to isolation and identification, cultures were performed on MacConkey agar in Petri dishes. The plates were incubated at 37°C overnight. Suspected colonies on this isolation medium (lactose-fermenting) were isolated and further characterized biochemically. All isolations and identifications were conducted according to the standard protocols (Rémic, 2007). *E. coli* isolates were sub-cultured on nutrient agar prior to antibiotic susceptibility testing. The antimicrobial susceptibility tests were performed by disc diffusion on Mueller Hinton agar, as recommended by the ‘‘Comité de l’Antibiogramme de la Société Française de Microbiologie’’ or, CA-SFM, in short (2011).

Table 1: Antibiotic concentrations for MIC testing (µg/mL)

Ampicillin	Tetracycline	Chloramphenicol
32	32	64
16	16	32
8	8	16
4	4	8
2	2	4
1	1	2
0.5	0.5	1
0.25	0.25	0.5
0.125	0.125	0.25

Expected MICs values for reference strains (CA-SFM 2011); *E. coli*: Ampicillin: ≤4 mg/L; Tetracycline: ≤4 mg/L; Chloramphenicol: ≤8 mg/L; *S. aureus*: Ampicillin: ≤0.125 mg/L; Tetracycline: ≤1 mg/L; Chloramphenicol: ≤8 mg/L

MIC testing: The MICs were assessed on two reference bacterial strains (*E. coli*: ATCC 25922 and *S. aureus:* QC 1625) for three antibiotics which are commonly found in the local market and can be provided by both formal (local pharmacies) and non formal (informal drug selling) networks in the setting. These antibacterial drugs included ampicillin (6 µg/10 IU), tetracycline (30 IU) and chloramphenicol (30 µg). The tests were repeated fifteen times by agar dilution. For each of these series of MIC testing, a new antibiotic specimen was required. Antimicrobials were dissolved in appropriate solvent (phosphate buffer saline for ampicillin, distilled water for tetracycline and chloramphenicol). Serial dilutions were prepared to obtain the agar concentrations displayed in the Table 1 provided. Bacterial suspensions equal to 0.5 McFarland were further diluted (1/100) to obtain working density. Spots of these suspensions were deposited on agar containing various concentrations of the antibiotics. These preparations were then incubated 24 h at 37°C. Upon revelation, the absence of visible growth at a given concentration of antibiotic was regarded as inhibition while the lowest concentration to which no bacterial growth was observed was recorded as the minimal inhibiting concentration.

Drug selling was investigated by moving to selling sites and expressing the desire to buy one for a health problem. The sellers could then give the names of different molecules they could provide. Availability for each drug was defined as a percentage by dividing the number of drug sellers that could provide a given antibiotic by the total number of drug sellers investigated.

Resistance frequencies observed in the laboratory, data from drug investigation and patient behavior were brought together and analyzed with Excel.

RESULTS

Data analysis revealed that about 95% of the participants had taken antimicrobials before they reported to the hospital. Out of these, 45% have used more than five different drugs; 12. 2% have taken four;

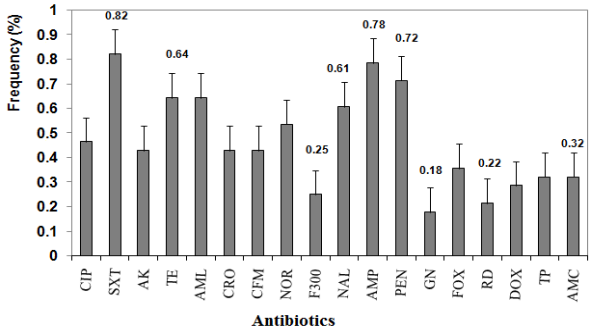

Fig. 1: Frequencies of resistant bacteria to various antibiotics

10.1% three and 10.6% more than one but could not remember the exact number in the past six months. Close to 16% recognized that they have taken traditional medicines while 6.1% have used both traditional medicines and conventional antimicrobials. With and without medical prescription, 84.3% bought their drugs from informal sellers.

A total of 35 drug sellers were identified (03 formal and 32 informal). When availability was assessed for conventional drugs, the most and the least available molecules included: co-tromoxazole 97.05%, amoxicillin 94%, ampicillin 85.29%, gentamycin 29.41%, rifampicin 0.03% and nitrofurantoin 0%. Availability was estimated for ciprofloxacin and norfloxacin at 70 and 3.1%, respectively.

Antibiotic susceptibility testing was performed on a total of 92 *E. coli* isolates, with 18 antibiotics. Figure 1 displays the frequencies of resistant bacteria.

This figure indicates that bacterial resistance phenotypes have developed against all antibiotics used in the present study. The highest frequency rates were observed with co-trimoxazole (82%), ampicillin (78%) and penicillin (72%). The three most active molecules were gentamycin, rifampicin and nitrofurantoin with resistance frequencies close to 18, 22 and 25%, respectively.

Resistance to more than one antibiotic was also common. Amoxicillin/ ampicillin (81%), amoxicillin/ penicillin (77%) and amoxicillin/amoxicillin-clavulanic acid (80%) were among the most frequently recorded. In addition, 98.2% of bacterial isolates that were resistant to norfloxacin were also resistant to ciprofloxacin.

Results obtained from MIC testing are summarized in Fig. 2, 3 and 4. Figure 2 displays the MIC values when chloramphenicol was used.

In both cases (*S. aureus* (2a) and *E. coli* (2b)), the MIC values fluctuated considerably. Most values were closer to the expected one (8 mg/L) when antibiotics provided by local pharmacies were used, compared to those obtained from informal sellers. This is illustrated by the tendency curves which reveal that the average MIC is lower with antibiotics provided by the formal distribution network. No Chloramphenicol concentration in the range prepared could inhibit *E. coli* growth during the 13th series of tests (>64 µg/mL).

Figure 3 displays variations of MIC values when tetracycline was used. For *S. aureus* (3a) and *E. coli* (3b) the average MIC values were higher with antibiotics from the informal network. These higher values were relatively more common with *E. coli* than *S. aureus* (10/15 *versus* 8/15), however.

For ampicillin, variations of the MIC values are presented in Fig. 4. In both *S. aureus* (4a) and *E. coli* (4b), the MICs were frequently higher for antibiotics from informal sector than the formal drug-selling networks. In some cases (tests number 6 for *E. coli* and number 2 for *S. aureus*), the MICs were about fourfold higher compared to the values observed with drugs from formal pharmacies.

DISCUSSION

This research revealed high frequencies of resistance to front-line antibiotics. The most reduced susceptibility was observed with thrimethoprim/ sulfam ethoxazol (co-trimoxazole) (82%). This combination

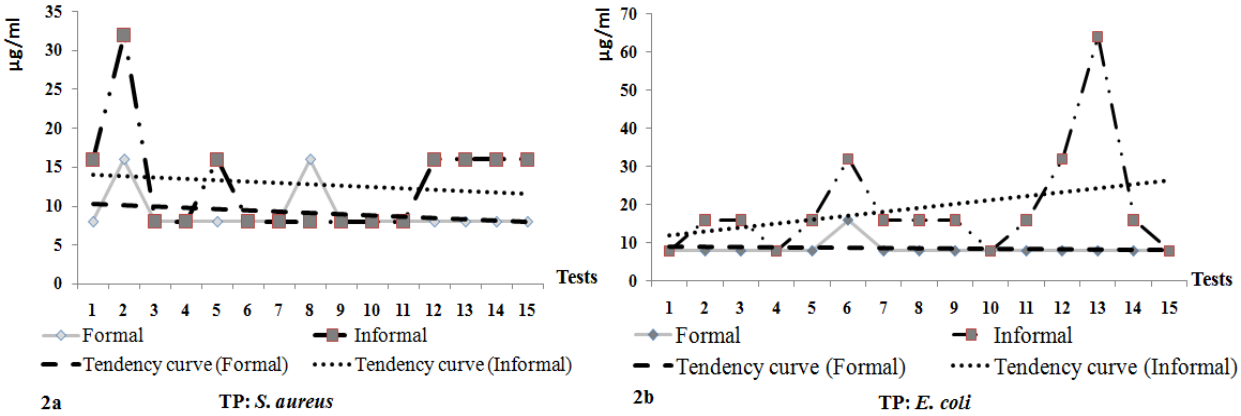

Fig. 2: Minimal inhibition concentration for (chloramphenicol 30 µg)

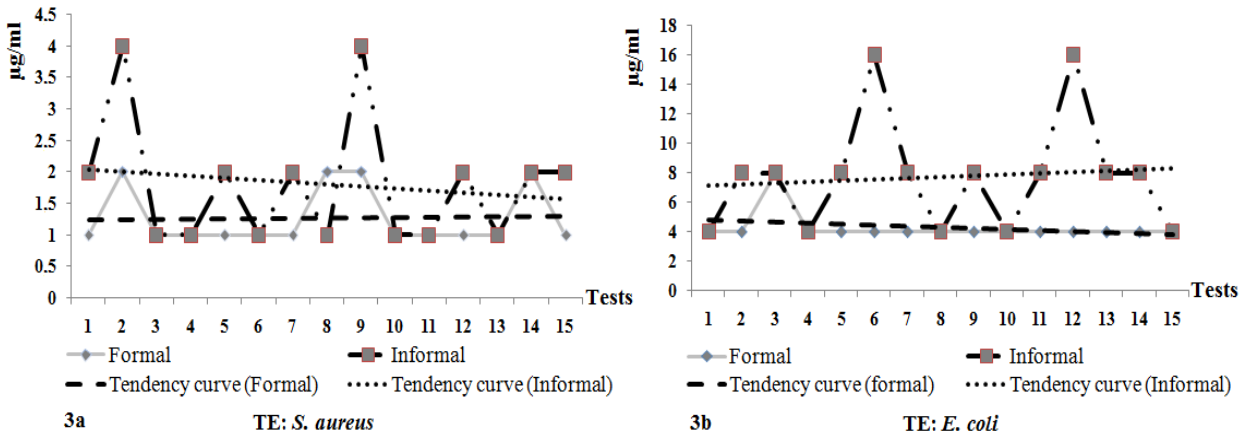

Fig. 3: Minimal inhibition concentration (tetracycline 30IU)

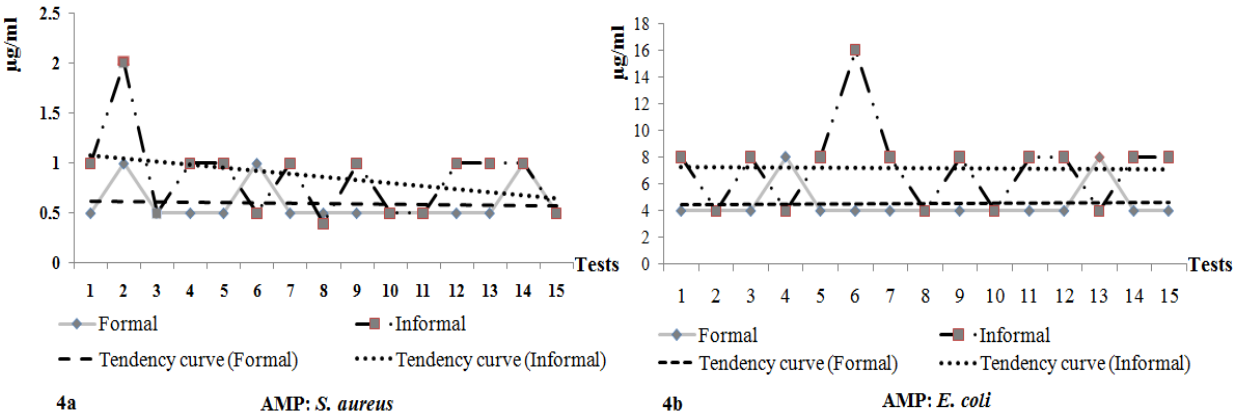

Fig. 4: Minimal inhibition concentration (ampicillin 10IU)

functions as anti-metabolites, blocking folic acid which is an essential compound in adenine and thymidine used in DNA and RNA synthesis. Other antimicrobials to which *E. coli* susceptibility was heavily reduced belong to the large group of penicillin (ampicillin 78%; penicillin 72%; amoxicillin 64%) and tetracycline (64%). These high rates of resistance can be explained, at least in part, by the fact that in Bangangté, self medication is common, like in most areas throughout the world where basic means of life are not provided. In addition, empirical therapy recommended by healthcare personnel is the rule since antimicrobial susceptibility testing cannot be performed prior to drug prescription. Accordingly, poverty in the setting likely associates with frequent chronic bacterial diseases as already observed by other authors (Wise *et al*., 1998; Fasehun, 1999; Cizman, 2003; Newman *et al*., 2011), due to inappropriate use of antibiotics that eventually selects resistant bacterial strains. Antibiotics which showed most reduced resistance rates included nitrofurantoin 25%, rifampicin 22% and gentamycin 18%. Drug availability followed a similar pattern and was estimated at 97, 94 and 85.29% for co-trimoxazole, amoxicillin, ampicillin; and 29.41, 0.03 and 0.0% for gentamycin, rifampicine and nitrofurantoin, respectively. Similar results were recorded with ciprofloxacin and norfloxacin, although resistance to norfloxacin was relatively less frequent (52 and 46%, respectively).

Another point this study highlighted was that antibiotics have frequently been used by the local populations prior to medical consultation. Assuming that availability and affordability result in frequent use, the resulting selection pressure exerted by drugs would also be responsible for frequent selection and cross-selection of resistant bacterial strains. This is consistent with the assertion that uncontrolled use of drugs is responsible for development and dissemination of resistance phenotypes in bacterial populations (Roger *et al*., 2003; Madingan and Martinko, 2007; Bisht *et al*., 2009). This assertion may be confirmed with the results obtained when co-trimoxazole and fluoroquinolones were used. Then, the relatively low resistance rates recorded with other antibiotics that are rarely found in the market like nitrofurantoin, gentamycin and rifampicin can be understood because they are not frequently used.

The frequency of resistance to more than one antibiotic was estimated at 85% in this investigation. Frequent multiple resistance (amoxicillin and ampicillin 81%, amoxicillin and penicillin 77%, for instance) is probably due to cross-resistance known to develop among drug analogues. In fact, amoxicillin (facilitated by oral administration) had probably selected resistance against ampicillin and penicillin (intramuscularly or intravenously administered). Recently, Newman *et al*. (2011) reported similar high frequencies of resistant bacteria in Ghana. Cross-resistance between ciprofloxacin and norfloxacin likely developed in a similar way, with ciprofloxacin (70% available) thought to select norfloxacin-resistant strains (98.2% of isolates that are resistant to ciprofloxacin are also resistant to norfloxacin). These trends were documented in *E. coli* isolated in other settings (Rigobelo *et al*., 2008; Edoh and Alomatu, 2008) and other bacterial species (Wagner *et al*., 2011).

Resistance growth to fluoroquinolones could actually be predicted in Cameroon because in the recent years, difficulties to manage bacterial infections with beta-lactams in clinical and non clinical settings had resulted in an increased empirical use of ciprofloxacin as first line antibiotic. As the living standard is low, monotherapy and treatment interruption (due to lack of financial means) are commonly reported. Major mechanisms involved in fluoroquinolone resistance include upregulation of efflux pumps, which export antimicrobials out of the bacterial cell (Gottesman *et al*., 2009) and mutations in the Quinolone Resistance-Determining Regions (QRDRs) of *gyrA* and *parC* (normally responsible for nucleic acid super-coiling), evidence that resistance to one fluoroquinolone may extend to the others (Namboodiri *et al*., 2011).

About 32% of bacterial isolates have developed resistance against beta-lactamase inhibitors (64% resistance to AML *versus* 32% to AMC). This is due to hydrolysis of the beta-lactam ring that results in inactivation of antibiotics from the beta-lactam family.

Basically, if the present survey targeted *E. coli*, data analysis and literature resources indicate that many other bacterial species may follow similar trends. The MIC could not be reached in some tests with the antibiotics dilution ranges used in this survey. In others, growth inhibition was observed when the quantities of drug used had largely exceeded those expected for appropriate concentration *in vivo* (MICx4). Furthermore, the tendency curves displayed the MIC values that were generally higher for antibiotics provided by the informal drug-selling networks. These findings are evidences that the active principles in these antibiotics have undergone potential reduction. Although the reason why the potential for these drugs becomes reduced is yet to elucidate (storage condition, for instance), it can be anticipated that any such event will result in low dosage *in vivo*, another key factor that promotes resistance growth, spread and persistent colonization by pathogenic bacteria.

Growth and spread of resistance phenotypes in bacteria are also reported to associate with the use of pesticides in agriculture and antibiotics for growth promotion in animal husbandry (Madingan and Martinko, 2007; Wagner *et al*., 2011). Many people in West Cameroon are farmers. Increased use of pesticides in plants and antibiotic-enriched feeds in farm animals that had developed steadily these recent years (unpublished data) may also explain the resistance observed with all categories of drug used in the present research.

New antimicrobials under development are more efficient in the management of bacterial infections than the current molecules (Poeppl *et al*., 2011; Fan *et al*., 2011). Some of these antibiotics have narrow spectra (Fan *et al*., 2011; Kim *et al*., 2011) with monotherapy, therefore, suitable for use in all areas. But if selection pressure caused by these molecules is minimized, they are more expensive and then, not affordably by people in resource-limited communities like most populations in Cameroon.

CONCLUSION

The present study disclosed high frequencies of resistant bacteria in Bangangté. From all indications, it appeared that bacterial resistance profiles were associated with the use of antibiotics (source of provision and drug utilization). Typically, informal drug-selling networks may represent a real threat to bacterial disease management and the whole healthcare system, just as drug consumption. These practices need to be discouraged through mass education on the consequences at individual and community levels. Drug susceptibility testing prior to antibacterial prescription should also be encouraged.

ACKNOWLEDGMENT

We are highly indebted to Doctor Marie-Christine Fonkoua (Centre Pasteur du Cameroon) who provided us with the reference bacterial strains used in the present survey.

REFERENCES

Andersson, D.I. and D. Hughes, 2011. Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol. Rev., 35: 901-911.

Bisht, R., A. Katiyar, R. Singh and P. Mittal, 2009. Antibiotic resistance: A global issue of concern. Asian J. Pharm. Clin. Res., 2: 34-39.

Chow, J.W., D.M. Shlaes, J.P. Quinn, D.C. Hoope, M.P. Johnson, *et al*., 1991. Enterrobacter bacteremia: Clinical features and emergence of antibiotic resistance during therapy. Ann. Intern. Med., 115: 585-590.

Cizman, M., 2003. The use and resistance of antimicrobials in the community. Int. J. Antimicrob. Agents, 21: 297-307.

Cohen, M.L., 1992. Epidemiology of drug resistance: Importance for a post antimicrobial era. Science, 257: 1050-1055.

Cuzon, G., T. Naas, M.V. Villegas, A. Correa, J.P. Quinn and P. Nordmann, 2011. Wide dissemination of Pseudomonas aeruginosa producing beta-Lactamase bla KPC-2 Gene in Colombia. Antimicrob. Agents Chemother., 55: 5350-5353.

Edoh, D. and B. Alomatu, 2008. Comparison of antibiotic resistance patterns between laboratories in Accra east Ghana. Niger. Annl. Nat. Sci., 8: 10-18.

Espinal, P., G. Fugazza, Y. López, M. Kasma, Y. Lerman, S. Malhotra-Kumar, H. Goossens, Y. Carmeli and J. Vila, 2011. Dissemination of an NDM-2-producing Acinetobacter baumannii clone in an Israeli rehabilitation center. Antimicrob. Agents Chemother., 55: 5396-5398.

Fan, Z., L. Cao, Y. He, J. Hu, Z. Di, Y. Wu, W. Li and Z. Cao, 2011. Ctriporin, A new anti-methicillin-resistant Staphylococcus aureus peptide from the venom of the scorpion chaerilus tricostatus. Antimicrob. Agents Chemother., 55: 5220-5229.

Fasehun, F., 1999. The antimicrobial paradox: Essential drugs effectiveness and cost. WHO, 77: 211-216.

Gold, H.S. and R.C. Moellering, 1996. Antimicribial-drug resistance. N. Engl. J. Med., 335: 1443-1445.

Gottesman, B.S., Y. Carmeli, P. Shitrit and M. Chowers, 2009. Impact of quinolone restriction on resistance patterns of Escherichia coli isolated from urine by culture in a community setting. Clin. Infect. Dis., 49: 869-875.

Jacob, M.E., J.T. Fox, S.L. Reinstein and T.G. Nagaraja, 2008. Antimicrobial susceptibility of foodborne pathogens in organic or natural production systems: An overview. Foodborne Pathog. Dis., 5: 721-730.

Kim, J.S., P. Heo, T.J. Yang, K.S. Lee, D.H. Cho, B.T. Kim, J.H. Suh, H.J. Lim, D. Shin, S.K. Kim and D.H. Kweon, 2011. Selective killing of bacterial persisters by a single chemical compound without affecting normal antibiotic-sensitive cells: Antimicrob. Agents Chemother, 55: 5380-5383.

Madingan, M.T. and J.M. Martinko, 2007. Biology of Microorganisms. 11th Edn., Pearson Education. Paris, France, pp: 1047, (French).

Malonza, I.M., M.A. Omari, J.J. Bwayo, A.K. Mwatha, A.N. Mutere, E.M. Murage and J.O. Ndinya-Achola, 1997. Community acquired bacterial infections and their antimicrobial susceptibility in Nairobi, Kenya. East Afr. Med. J., 74(3): 166-170.

McGowan Jr., J.E. 1983. Antimicrobial resistance in hospital organisms and its relation to antibiotic use. Rev. Infect Dis., 5: 1033-1048.

Namboodiri, S.S., J.A. Opintan, R.S. Lijek, M.J. Newman and I.N. Okeke, 2011. Quinolone resistance in Escherichia coli from Accra. BMC Microbiol, Ghana, 11: 44.

Newman, M.J., E. Frimpong, E.S. Donkor, J.A. Opintan and A. Asamoah-Adu, 2011. Resistance to antimicrobial drugs in Ghana. Infect Drug Resis., 4: 215-220.

Poeppl, W., S. Tobudic, T. Lingscheid, R. Plasenzotti, N. Kozakowski, H. Lagler, A. Georgopoulos and H. Burgmann, 2011. Daptomycin, fosfomycin, or both for treatment of methicillin-resistant Staphylococcus aureus osteomyelitis in an experimental rat model. Antimicrob. Agents Chemother., 55: 4999-5003.

Rémic, 2007. Repository in Medical Microbiology (Bacteriology and Mycology). 3rd Edn., SFM, Paris. Vivactis Plus, Paris, France, pp: 232.

Rigobelo, E.C., E. Santo and J.M. Marin, 2008. Beef carcass contamination by shiga toxin-producing Escherichia coli strains in abattoir in Brazil: Characterization and resistance to antimicrobial drugs. Foodborne Pathog. Dis., 5: 811-817.

Roger, F.G., D. Greenwood, S.R. Norrbby and R.J. Whistley, 2003. Antibiotic and Chemotherapy, the Problem of Resistance. 8th Edn., Churchill Livingstone, pp: 25-47.

Shetty, N., J.W. Tang and J. Andrews, 2009. Infectious Diseases Pathogenesis, Prevention and Case Studies. 1st Edn., Blackwell, UK, pp: 691.

Wagner, D.R., J.J. Shemedia, E.C. Carl and D.E. Bruce, 2011. Bovine intestinal bacteria inactivate and degrade ceftiofur and ceftriaxone with multiple beta-lactamases. Antimicrob. Agents Chemother., 55: 4990-4998.

WHO, 2002. Fact Sheet N°194 Revised. Retrieved from: http// www. who. nt/ mediacenter/ factshets/ fs194/ en, (Accessed on: July 14, 2012).

WHO, 2011. Antimicrobial Resistance. Retrieved from: www.who.int/world-health-day/2011, (Accessed on: July 14, 2012).

Wise, R., T. Hart, O. Cars, M. Streulens, R. Helmuth, P. Huovinen and M. Sprenger, 1998. Antimicrobial resistance is a major threat to public health. BMJ, 317: 609-610.

Zhang, R., K. Eggleston, V. Rotimi and R.J. Zeckhauser, 2006. Antibiotic resistance as a global threat: Evidence from China, Kuwait and the United States. Global Health, 2: 6.