International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 4, Issue 2, pp: (560-566), Month: October 2016 - March 2017, Available at: www.researchpublish.com

Overview of Antibiotics Resistance Crisis and Its Management

[1]Feras Jamal H Alwaznah, [2]Ra'ad Omar Alhelfawi, [3]Moatasem Mohammed Modhish, [4]Salem Ahmed Alghamdi, [5]Musaab Khalil Albalawi, [6]Fares jamal H alwaznah

Abstract: **The development of drug-resistant pathogens is a significant obstacle for 21st century medication. Substance abuse practices strongly promoted as resistance management tools by expert bodies, public health firms. The aim of this overview was to highlight the most important aspects about antibiotics resistance and the impact of this crisis on healthcare system from general practice to dental care. also we attempted to discuss the control and management approaches of antibiotics crisis. We conducted a comprehensive review of literature concerning antibiotics resistance through electronic search in several databases; MIDLINE, EMBASE, GOOGLE Scholar, up to December 2016. We identified most important studies that discussing the impact of antibiotic resistance on healthcare and approaches to control this crisis, we limited our search to English language, we also involve animal trails in this review. Antibiotic resistance genes occurred long ago in action to naturally happening prescription antibiotics. Modern medication has actually owned even more advancement of a few of these genes. Resistance can likewise develop spontaneously by anomaly. In germs, genes can be acquired or they can be gotten from non-relatives on mobile hereditary aspects like plasmids. This horizontal gene transfer can happen in between extremely various germs.**

Keywords: **Healthcare System, Modern Medication Antibiotics Resistance, Antibiotics Crisis.**

1. INTRODUCTION

Antibiotics in the biosphere are produced by microorganisms as secondary metabolites at a concentration much lower than the therapeutic dosage. Waksman was convinced that antibiotics play "no genuine part in influencing or customizing living processes that take place in nature" [(1)] though there is evidence to the contrary [(2)]. Antibiotics are produced in the late stages of microbial fixed development stage, decoupled from the doubling time, indicating that they are not indispensable for sustenance of life of the producer organism. However, it is likewise a truth that the production of an antibiotic is a multi-step process that includes a number of genes. It does not appear tenable that such a complex anabolic process has actually been sustained through development without having any obvious function to serve. Current research studies [(3,4,5,6)] reveal that antibiotics do have some particular results on the natural scene of the microbes while they presume a totally different role as antibacterial agents in the dose utilized in rehabs [(3,4,5,6)].

Antibiotic resistance is 'germs changing in ways that remove the efficiency or decrease of antibiotics' [(9)]. These changes are due to bacterial advancement, and threaten the single greatest therapeutic advance in the history of medication. The quick emergence of resistant germs is happening worldwide, endangering the efficacy of antibiotics, which have changed medication and saved millions of lives [(5,6,7,8)]. Many years after the very first patients were treated with prescription antibiotics, bacterial infections have again end up being a threat [(8)]. The antibiotic resistance crisis has actually been attributed to the overuse and abuse of these medications, along with a lack of new drug development by the pharmaceutical industry due to lowered financial incentives and tough regulative requirements [(9,10,11)].

The objective of resistance management is to prevent medical failures caused by top-level resistance. Resistance is often a continuous trait, and there can be differing degrees of intermediate resistance. Often described as "tolerance," intermediate resistance provides the ability to make it through concentrations of drug below those thought about healing (**Figure 1**) [(12)].

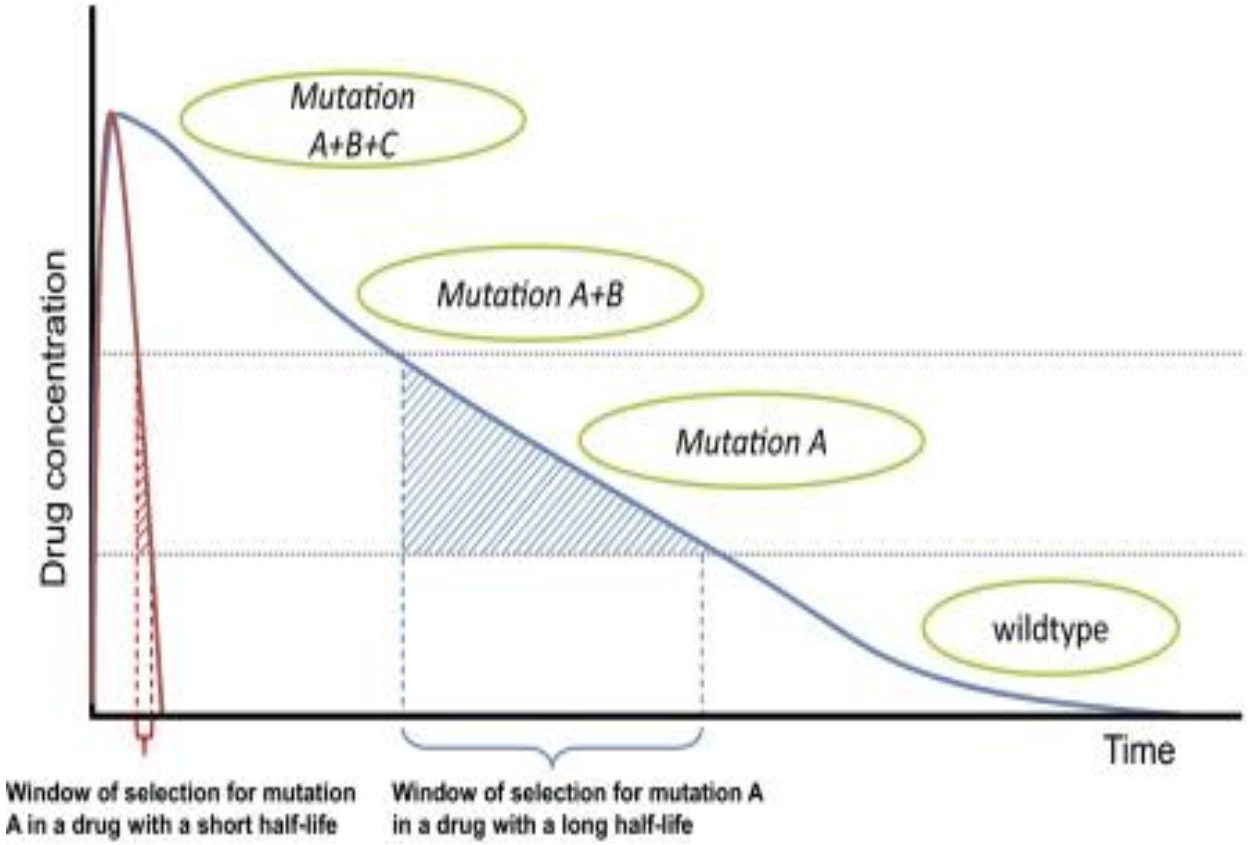

Figure1: Hypothetical path to drug resistance. Solid curves show drug concentration in a treated patient for two drugs with different half-lives; concentrations wane when treatment ceases. In this schematic, wild type parasites can survive very low concentrations, with mutations A, B, and C conferring the ability to survive ("tolerate") successively higher drug concentrations [12].

The aim of this overview was to highlight the most important aspects about antibiotics resistance and the impact of this crisis on healthcare system from general practice to dental care. also we attempted to discuss the control and management approaches of antibiotics crisis.

2. METHODOLOGY

We conducted a comprehensive review of literature concerning antibiotics resistance through electronic search in several databases; MIDLINE, EMBASE, GOOGLE Scholar, up to December 2016. We identified most important studies that discussing the impact of antibiotic resistance on healthcare and approaches to control this crisis, we limited our search to English language, we also involve animal trails in this review.

3. RESULT AND DISSCUSION

History of antibiotics and mode of resistance action:

Since the established in 1937 of the first reliable antimicrobials, namely, the sulfonamides, the development of particular systems of resistance has plagued their therapeutic usage. Sulfonamide resistance was initially reported in the late 1930s, and the very same systems operate some 70 years later on [13]. A collection of the frequently utilized prescription antibiotics, their modes of action, and resistance systems is shown in (**Table 1**) [14]. Penicillin was found by Alexander Fleming in 1928, and in 1940, numerous years before the intro of penicillin as a therapeutic, a bacterial penicillinase was determined by two members of the penicillin discovery team [13]. When the antibiotic was used widely, resistant strains capable of inactivating the drug ended up being widespread, and synthetic research studies were carried out to modify penicillin chemically to prevent cleavage by penicillinases (β-lactamases). Remarkably, the recognition of a bacterial penicillinase prior to using the antibiotic can now be appreciated in the light of recent findings that a large number of antibiotic r genes are components of natural microbial populations [15].

Table1: Modes of action and resistance mechanisms of commonly used antibiotics [14]

Antibiotic class	Example(s)	Target	Mode (s) of resistance
β-Lactams	Penicillins (ampicillin), cephalosporins (cephamycin), penems (meropenem), monobactams (aztreonam)	Peptidoglycan biosynthesis	Hydrolysis, efflux, altered target
Aminoglycosides	Gentamicin, streptomycin, spectinomycin	Translation	Phosphorylation, acetylation, nucleotidylation, efflux, altered target
Glycopeptides	Vancomycin, teicoplanin	Peptidoglycan biosynthesis	Reprogramming peptidoglycan biosynthesis
Tetracyclines	Minocycline, tigecycline	Translation	Monooxygenation, efflux, altered target
Macrolides	Erythromycin, azithromicin	Translation	Hydrolysis, glycosylation, phosphorylation, efflux, altered target
Lincosamides	Clindamycin	Translation	Nucleotidylation, efflux, altered target
Streptogramins	Synercid	Translation	C-O lyase (type B streptogramins), acetylation (type A streptogramins), efflux, altered target
Oxazolidinones	Linezolid	Translation	Efflux, altered target
Phenicols	Chloramphenicol	Translation	Acetylation, efflux, altered target
Quinolones	Ciprofloxacin	DNA replication	Acetylation, efflux, altered target
Pyrimidines	Trimethoprim	C_1 metabolism	Efflux, altered target
Sulfonamides	Sulfamethoxazole	C_1 metabolism	Efflux, altered target
Rifamycins	Rifampin	Transcription	ADP-ribosylation, efflux, altered target
Lipopeptides	Daptomycin	Cell membrane	Altered target
Cationic peptides	Colistin	Cell membrane	Altered target, efflux

Benefits of Antibiotics:

Prescription antibiotics have not just conserved patients' lives, they have played a critical function in attaining major advances in medication and surgical treatment [6]. They have actually effectively prevented or treated infections that can happen in patients who are receiving chemotherapy treatments; who have persistent diseases such as diabetes, end-stage kidney disease, or rheumatoid arthritis; or who have had complex surgeries such as organ transplants, joint replacements, or cardiac surgical treatment [6,7,9].

Prescription antibiotics have likewise assisted to extend expected life spans by changing the outcome of bacterial infections [20,21]. In 1920, individuals in the United States were anticipated to live to be only 56.4 years old; now, however, the typical U.S. life expectancy is almost 80 years [9]. Prescription antibiotics have actually had comparable helpful results worldwide. In developing countries where sanitation is still bad, prescription antibiotics decrease the morbidity and mortality caused by other and food-borne poverty-related infections [21].

Mechanism of antibiotics resistance:

molecular mechanism systems of resistance to prescription antibiotics have actually been studied thoroughly (**Table 1**) and have actually included examinations of the genetics and biochemistry of various elements of bacterial cell function [16,17,18]. The research study of antibiotic action and resistance has actually contributed considerably to our understanding of cell structure and function. Resistance procedures are commonly dispersed in the microbial kingdom and have actually been well explained for a range of commensals [19] and pathogens; most can be shared by several unique gene transfer systems. A few of the resistance types that show the problems in keeping efficient antibiotic activity in the face of the biochemical and hereditary versatility of germs are worthy of unique reference.

Intrinsic Resistance:

Intrinsic resistance describes the presence of genes in bacterial genomes that might create a resistance phenotype, i.e., proto- or quasi-resistance. Various genera, types, pressures, and so on, display varieties of antibiotic reaction phenotypes. Given that the start of this millennium, the schedule of genomewide mutagenesis strategies and quick bacterial genome sequencing has actually exposed lots of potential/intrinsic gene functions in germs that might cause resistance phenotypes in scientific circumstances. A typical hereditary path to boosted antibiotic resistance is gene amplification, significantly for resistance to the sulfonamides [22] and trimethoprim [23]. These research studies offer excellent ideas regarding exactly what might occur in the future.

Antibiotic resistance in Gram-positive pathogens:

A crisis however still under control Among Gram-positive pathogens, Staphylococcus aureus and Enterococcus spp. are the types which presently posture the significant obstacles in regards to antibiotic resistance. Methicillin-resistant S. aureus (MRSA), emerged because 5 years, has actually been the very first significant gamer in the antibiotic resistance crisis, showing a worldwide diffusion and a considerable effect on medical results versus methicillin-susceptible S. aureus [24,25,26]. The MRSA phenotype is because of the expression of customized penicillinbinding proteins (PBPs), encoded by the horizontally gotten mec genes, that take control of the functions of the resident staphylococcal PBPs and are not prevented by traditional b-lactams. MRSA rates are rather high in a number of nations in Europe, the Americas and the AsiaPacific area, where MRSA stays a crucial reason for human infections [27,28,29]. In some nations, nevertheless, aggressive infection control projects have actually shown effective at avoiding MRSA dissemination (e.g. in the Netherlands) [27] or at suppressing a currently developed MRSA endemicity (e.g. in the United Kingdom) [27,30], showing that infection control can be extremely reliable at restricting MRSA dissemination. On the other hand, there are still a variety of drugs which maintain activity versus MRSA, consisting of the glycopeptides (e.g. vancomycin and teicoplanin), linezolid, tigecycline, daptomycin as well as some brand-new b-lactams, such as ceftaroline and ceftobiprole, that are active versus the customized PBPs responsible for the methicillin-resistant phenotype [31]. Resistance to any of these drugs has actually been reported, the resistance rates stay general extremely low [32,33,34], while XDR or TDR MRSA stress have actually not been regularly reported.

Antibiotic resistance in Gram-negative pathogens:

A crisis going out of control with Gram-negative pathogens the antibiotic crisis is presently more major than with the Gram-positives. The event of XDR and even TDR phenotypes has actually been regularly reported amongst Gram-negative pathogens associated with HAIs, such as Pseudomonas aeruginosa, Acinetobacter spp. and Enterobacteriaceae (primarily Klebsiella pneumoniae) [35,36,37]. On the other hand, MDR Gram-negatives are progressively common likewise in the neighborhood, consisting of Escherichia coli producing extended-spectrum beta-lactamases (ESBLs) [38,39], and Neisseria gonorrhoeae resistant to fluoroquinolones, tetracycline, azithromycin and penicillin or expanded-spectrum cephalosporins [40]. P. aeruginosa has actually likely been the very first pathogen to show MDR and XDR phenotypes, with the development of pressures resistant to all classes of anti-pseudomonal representatives other than polymyxins (likewise called Colistin-Only Susceptible-COS- stress). MDR and XDR pressures of P. aeruginosa are discovered as agents of high-risk clones belonging in global clonal family trees, such as ST111, st175 and st235 [41].

Management approaches of antibiotics resistance:

Logical advancement of treatment routines that provide reliable resistance management needs a sound knowledge base [42,43,44], and there is significant scope for examining the evolutionary effects of various treatment programs for a wide variety of diseases. Preferably, these would include quantitative contrasts of how contrasting programs impact each of the goals of patient treatment: resistance, infectiousness, and health management. In concept, such research studies can be done on animal designs [45,46,47] and, in a more restricted method, on human beings [48]. It is possible to determine the evolutionary effects of contending resistance management techniques in healthcare facilities [49,50,51], and it may even be possible in human neighborhoods. Penilla et al. [52] arbitrarily assigned 24 towns in Mexico to among 4 various techniques of using public health insecticides and compared the rate of increase of resistant mosquitoes over a number of years. None of the putative resistance management methods slowed the spread of phenotypic resistance.

4. CONCLUSION

The value and worth of prescription antibiotics cannot be overstated; we are absolutely depending on them for the treatment of contagious diseases, and they must never ever be thought about simple products. In addition to their usage in the treatment of contagious diseases, prescription antibiotics are vital to the success of innovative surgeries, consisting of organ and prosthetic transplants. Regardless of all great intents to manage antibiotic use (however restricted action), there is little doubt that the scenario with respect to antibiotic resistance is grim. Resistance systems are pandemic and produce a huge medical and monetary concern on healthcare systems worldwide. There are no basic services to the issue. Definitive actions that need substantial dedication and enforcement are never ever popular, even if lives can be conserved. Not all bacterial pathogens are resistant all of the time, and lots of react to empirical treatment with antimicrobial representatives administered in the neighborhood. Some brand-new drugs with activity versus MDR pathogens, consisting of CRE, are presently discovered in the sophisticated phases (Phases 2 and 3) of the antibiotic pipeline (e.g. plazomicin, ceftazidime-avibactam and other mixes in between b-lactams and brand-new b-lactamase inhibitors, eravacycline. The intro of these brand-new substances in scientific practice is anticipated no faster than 3-- 5 years. Awaiting these brand-new drugs, the only choices that are presently offered to manage the antibiotic resistance crisis are represented by: enhancing habits focused on decreasing the dissemination of XDR pathogens (based upon monitoring, infection control practices and minimizing unneeded antimicrobial pressure by antimicrobial stewardship programs); and optimization of the readily available antimicrobial treatment programs by choice of the most efficient dosing routines and mixes.

REFERENCES

[1] Waksman S. A. (1961). The role of antibiotics in nature. *Perspect. Biol. Med.* 4 271–286

[2] Gullberg E., Cao S., Berg O G., Illback C., Sandegren L., Hughes D., et al. (2011). Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog*. 7:e100215810.1371/journal.ppat.1002158

[3] Davies J. (2009). Darwin and microbiomes. *EMBO Rep.* 10 805

[4] Ventura M., Canchaya C., Tauch A., Chandra G., Fitzgerald G. F., Chater K. F., et al. (2007). Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiol. Mol. Biol. Rev.* 71 495–548

[5] Golkar Z, Bagazra O, Pace DG. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. J Infect Dev Ctries. 2014;8(2):129–136. 13.

[6] Gould IM, Bal AM. New antibiotic agents in the pipeline and how they can overcome microbial resistance. Virulence. 2013;4(2):185–191.

[7] Wright GD. Something new: revisiting natural products in antibiotic drug discovery. Can J Microbiol. 2014;60(3):147–154.

[8] Sengupta S, Chattopadhyay MK, Grossart HP. The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol. 2013;4:47.

[9] Centers for Disease Control and Prevention, Office of Infectious Disease Antibiotic resistance threats in the United States, 2013. Apr, 2013. Available at: http://www.cdc.gov/drugresistance/threat-report-2013.

[10] Spellberg B, Gilbert DN. The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. Clin Infect Dis. 2014;59 (suppl 2):S71–S75.

[11] Viswanathan VK. Off-label abuse of antibiotics by bacteria. Gut Microbes. 2014;5(1):3–4.

[12] Read AF, Day T, Huijben S. The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(Suppl 2):10871-10877. doi:10.1073/pnas.1100299108.

[13] Abraham, E. P., and E. Chain. 1940. An enzyme from bacteria able to destroy penicillin. Rev. Infect. Dis. 10**:**677-678.

[14] Wright, G. D., and M. Morar. The genomic enzymology of antibiotic resistance. Annu. Rev. Genet., in press.

[15] D'Costa, V. M., K. M. McGrann, D. W. Hughes, and G. D. Wright. 2006. Sampling the antibiotic resistome. Science 311**:**374-377.

[16] Allen, H. K., J. Donato, H. H. Wang, K. A. Cloud-Hansen, J. E. Davies, and J. Handelsman. 2010. Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. 8**:**251-259.

[17] Gale, E. F., E. Cundliffe, P. E. Reynolds, M. H. Richmond, and M. J. Waring (ed.). 1981. The molecular basis of antibiotic action, 2nd ed. John Wiley, Chichester, United Kingdom.

[18] Walsh, C. 2003. Antibiotics: actions, origins, resistance. ASM Press, Washington, DC.

[19] Marshall, B. M., D. J. Ochieng, and S. B. Levy. 2009. Commensals: unappreciated reservoir of antibiotic resistance. Microbe 4**:**231-238.

[20] Piddock LJ. The crisis of no new antibiotics—what is the way forward? Lancet Infect Dis. 2012;12(3):249–253.

[21] Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. Clin Opin Pharmacol. 2014;18:56–60.

[22] Kashmiri, S. V. S., and R. D. Hotchkiss. 1975. Evidence of tandem duplication of genes in a merodiploid region of pneumococcal mutants resistant to sulfonamide. Genetics 81**:**21.

[23] Brochet, M., E. Couvé, M. Zouine, C. Poyart, and P. Glaser. 2008. A naturally occurring gene amplification leading to sulfonamide and trimethoprim resistance in *Streptococcus agalactiae*. J. Bacteriol.190**:**672-680.

[24] WHO (World Health Organization): Antimicrobial Resistance: Global Report on surveillance. 2014 http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf.

[25] Boucher H, Miller LG, Razonable RR: Serious infections caused by methicillin-resistant Staphylococcus aureus. Clin Infect Dis 2010, 51(Suppl. 2):S183-S197.

[26] Hanberger H, Walther S, Leone M, Barie PS, Rello J, Lipman J, Marshall JC, Anzueto A, Sakr Y, Pickkers P et al.: Increased mortality associated with methicillin-resistant Staphylococcus aureus (MRSA) infection in the intensive care unit: results from the EPIC II study. Int J Antimicrob Agents 2011, 38:331-335.

[27] ECDC: European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2012. Annual Report of the European Antimicrobial Resistance Surveillance Network. 2013.

[28] David MZ, Daum RS, Bayer AS, Chambers HF, Fowler VG, Miller LG, Ostrowsky B, Baesa A, Boyle-Vavra S, Eells SJ et al.: Staphylococcus aureus bacteremia at five U.S. academic medical centers, 2008–2011: significant geographic variation in community-onset infections. Clin Infect Dis 2014 http:// dx.doi.org/10.1093/cid/ciu410.

[29] Chen C-J, Huang Y-C: New epidemiology of Staphylococcus aureus infection in Asia. Clin Microbiol Infect 2014:1270 http:// dx.doi.org/10.1111/1469-0691.5.

[30] Johnson AP, Davies J, Guy R, Abernethy J, Sheridan E, Pearson A, Duckworth G: Mandatory surveillance of methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia in England: the first 10 years. J Antimicrob Chemother 2012, 67:802-809.

[31] Kurosu M, Siricilla S, Mitachi K: Advances in MRSA drug discovery: where are we and where do we need to be? Expert Opin Drug Discov 2013, 8:1095-1116. 14. Sader HS,

[32] Farrell DJ, Flamm RK, Jones RN: Daptomycin activity tested against 164457 bacterial isolates from hospitalised patients: summary of 8 years of a Worldwide Surveillance Programme (2005–2012). Int J Antimicrob Agents 2014, 43:465-469.

[33] Mendes RE, Deshpande LM, Jones RN: Linezolid update: stable in vitro activity following more than a decade of clinical use and summary of associated resistance mechanisms. Drug Resist Updat 2014 http://dx.doi.org/10.1016/ j.drup.04.002.

[34] Alm RA, McLaughlin RE, Kos VN, Sader HS, Iaconis JP, Lahiri SD: Analysis of Staphylococcus aureus clinical isolates with reduced susceptibility to ceftaroline: an epidemiological and structural perspective. J Antimicrob Chemother 2014 http:// dx.doi.org/10.1093/jac/dku114.

[35] Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B et al.: Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012, 18:268-281.

[36] Livermore DM: Current epidemiology and growing resistance of Gram-negative pathogens. Korean J Intern Med 2012, 27:128-142.

[37] Pendleton JN, Gorman SP, Gilmore BF: Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther 2013, 11:297-308.

[38] Pitout JDD: Enterobacteriaceae that produce extendedspectrum b-lactamases and AmpC b-lactamases in the community: the tip of the iceberg? Curr Pharm Des 2013, 19:257-263.

[39] Woerther P-L, Burdet C, Chachaty E, Andremont A: Trends in human fecal carriage of extended-spectrum b-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev 2013, 26:744-758.

[40] Unemo M, Nicholas RA: Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. Future Microbiol 2012, 7:1401-1422.

[41] Woodford N, Turton JF, Livermore DM: Multiresistant Gramnegative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev 2011, 35:736-755.

[42] zur Wiesch PA, Kouyos R, Engelstädter J, Regoes RR, Bonhoeffer S. Population biological principles of drug-resistance evolution in infectious diseases. Lancet Infect Dis. 2011;11:236–247.

[43] Read AF, Huijben S. Evolutionary biology and the avoidance of antimicrobial resistance. Evol Appl. 2009;2:40–51.

[44] Goncalves BPA, Paul REL. Sub-clearance treatment to slow malaria drug resistance? Trends Parasitol. 2011;27:50–51.

[45] de Roode JC, Culleton R, Bell AS, Read AF. Competitive release of drug resistance following drug treatment of mixed *Plasmodium chabaudi* infections. Malar J. 2004;3:33.

[46] Wargo AR, Huijben S, de Roode JC, Shepherd J, Read AF. Competitive release and facilitation of drug-resistant parasites after therapeutic chemotherapy in a rodent malaria model. Proc Natl Acad Sci USA. 2007;104:19914–19919.

[47] Huijben S, et al. Chemotherapy, within-host ecology and the fitness of drug-resistant malaria parasites. Evolution. 2010;64:2952–2968.

[48] Harrington WE, et al. Competitive facilitation of drug-resistant *Plasmodium falciparum* malaria parasites in pregnant women who receive preventive treatment. Proc Natl Acad Sci USA. 2009;106:9027–9032.

[49] Brown EM, Nathwani D. Antibiotic cycling or rotation: A systematic review of the evidence of efficacy. J Antimicrob Chemother. 2005;55:6–9.

[50] Martínez JA, et al. Comparison of antimicrobial cycling and mixing strategies in two medical intensive care units. Crit Care Med. 2006;34:329–336.

[51] Smith RL, et al. Reduction in rates of methicillin-resistant *Staphylococcus aureus* infection after introduction of quarterly linezolid-vancomycin cycling in a surgical intensive care unit. Surg Infect (Larchmt) 2008;9:423–431.

[52] Penilla RP, et al. Cytochrome P450-based resistance mechanism and pyrethroid resistance in the field *Anopheles albimanus* resistance management trial. Pestic Biochem Physiol. 2007;89:111–117.