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Assessing Pharmaceutical Equivalence of Generic Antibiotics Using *in vitro* Antimicrobial Susceptibility of Some Hospital Strains in Rwanda

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JNK and CIK designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JBN managed the literature searches of the study. Author AM managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

The study aimed at evaluating the pharmaceutical equivalence of generic commercial products containing same common antibiotics by testing *in vitro* the antimicrobial susceptibility/resistance of common bacterial isolates from patients. In total 35 different generic preparations and brands corresponding to seven antibiotics- Ampicillin, Amoxicillin, Amoxicillin/clavulanic acid, Cotrimoxazole, Norfloxacin, and Erythromycin- were compared by a disc diffusion method against three pathogenic strains- *Salmonella typhi, Shigella flexneri and Staphylococcus aureus*- isolated from patients. Some brands were presumptively regarded as good quality medicines to serve as gold standards instead of using international references. The pharmaceutical quality of the

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preparations was checked by visual inspection and identification of active ingredients according to referral pharmacopeias. All products satisfied visual inspection and identification tests. However they exhibited differences in their antimicrobial profiles and potency. Two generic preparations containing amoxicillin/clavulanic acid were out of specifications (<90%) as compared with Augmentin® gold standard. Comparing the susceptibility of bacteria by the diameter (d) of inhibition zone in mm, *Salmonella typhi* was susceptible to Norfloxacin (d=23.2), low to Augmentin (d=11.5), and resistant to Ampicillin, Amoxicillin, Cotrimoxazole and Erythromycin (d=0). *Shigella flexneri* was susceptible to all antibiotics (d=31.6 – 42.8) except Erythromycin (d=0) which exhibited the lowest spectrum of activity. *Staphylococcus aureus* was susceptible to all antibiotics with different potencies (d=20 for Amoxicillin – d=42.6 for Norfloxacin). These findings showed the possibility of using a simple *in vitro* antimicrobial susceptibility testing to compare the equivalence of marketing antibiotic products in quality and efficacy. The result also could help clinicians choosing the most appropriate antibiotic in treatment.

Keywords: Antimicrobial susceptibility; resistance; Shigella; Staphylococcus; Salmonella; antibiotics.

1. INTRODUCTION

The spread of multiple antimicrobial-resistant pathogenic bacteria has been reported by the World Organization for Animal Health (OIE), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a serious global human and animal health problem [1]. Cumulative data have stressed that drug-resistant pathogens are a growing menace to all people, regardless of age, gender, or socioeconomic background; they also endanger people in affluent industrial societies like the United States as well as in less-developed nations [2-5]. Numerous studies have furthermore underlined a big concern about the circulation of poor quality medicines in the world, particularly in Sub-Saharan countries where monitoring and quality assessment systems are unsatisfactory even though no country worldwide is totally spared [6-9]. Amid many counterfeit medicines reported to the WHO each year, the highest percentage concerns antimicrobials [2-5]. The use of ineffective or poor quality antimicrobial drugs has multiple drawbacks leading to microbial resistance, therapeutic failure, exacerbation of disease, and increasing death rates [6-9]. In pharmacy practice, there is indeed a constantly mandatory need to ensure that every marketing pharmaceutical preparation complies with regulatory requirements to allow detecting poor quality medicine and avoid such kind of drawbacks and harms to patients [1]. Further to studies showing a high extent of microbial resistance in Eastern Africa Region [10-13] and particularly in Rwanda [14-17], we undertook this study to highlight the current situation about the quality and efficacy equivalence of antibiotics circulating on the market, hoping the result will help clinicians to choose the most appropriate antibiotics and the authority to take appropriate actions.

2. MATERIALS AND METHODS

2.1 Antibiotics Samples

In total, 35 products presented in uncoated or coated tablets and capsules from different countries and corresponding to seven essential antibiotics (Ampicillin, Amoxicillin, Augmentin, Chloramphenicol, Cotrimoxazole, Erythromycin, and Norfloxacin) were collected. Four generic samples of each antibiotic were randomly sampled. One part of the antimicrobial products was purchased from private pharmaceutical drugstores in Kigali and another part was kindly obtained from former Laboratoire Pharmaceutique (LABOPHAR) and from Central d'Achat des Medicaments Essentiels du Rwanda (CAMERWA). Some brands were presumptively regarded as good quality medicines to serve as gold standard for the analysis of generic preparations (Table 1).

2.2 Pharmaceutical Quality Tests

The evaluation consisted only with identification tests by visual inspection and chemical reactions. The material and reagents were prepared as described in the referential pharmacopoeias (British pharmacopeia; European pharmacopeia; United States Pharmacopoeia). Tests were performed in the Quality Control Laboratory of LABOPHAR.

2.3 Bacterial Strains and Seeding Medium

Salmonella typhi, Shigella flexneri and Staphylococcus aureus were isolated from

patients at the Butare University Teaching Hospital (BUTH). Some strains were randomly picked up and cultured on Mueller-Hinton Agar (MHA SS Park Scientific, 60 g/L) for *Salmonella/Shigella* and Mannitol salt agar (MHA MS bioLab, 110 g/L) for *Staphylococcus aureus*. The seeding medium for antimicrobial susceptibility test (AST) was prepared according to the manufacturer's instructions from a commercial dehydrated base (bioLab). For 1 liter of culture medium, 38 g Mueller-Hinton was mixed with 1 liter of purified water and heated until boiling. The medium was then autoclaved at 121℃ for 15 minutes. Immediately after autoclaving, it was cooled in a 45 to 50°C water bath. The freshly prepared and cooled medium was poured into glass or plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponded to 30 ml for plates with a diameter of 100 mm. The agar medium was allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C). A representative sample of each batch of plates was examined for sterility by incubating at 30 to 35°C for 48 hours.

2.4 Antimicrobial Susceptibility Testing

The AST was carried out in the Microbiological Lab of LADAMET in Butare using a disc diffusion Kirby-Bauer method according to Clinical and Laboratory Standards Institute (CLSI) [18-22]. As routine control in the Laboratory, the quality of the medium is often checked with Staphylococcus ATCC® 25923. Whatman filter paper no1 was used to prepare discs approximately 6 mm in diameter, which were placed in a Petri dish and sterilized in a hot air oven. Antibiotic products were powdered and aliquots dissolved in appropriate solvents according to the pharmacopoeias' instruction and then diluted up to the desired concentration which is the minimum inhibitory concentration of each antibiotic [18]. The loop used for delivering the antibiotics was made of 20 gauge wire and had a diameter of 2 mm. This delivers 0.005 ml of antibiotics to each disc. The method was first validated for linearity in inhibitory zones using 3 dose levels of Ampicillin products. The mean inoculums sizes were 1.610^{8}CFU/ml to 2.910^{8}CFU/ml. Fig. 1 illustrates the Petri dish plates used and inhibition zones.

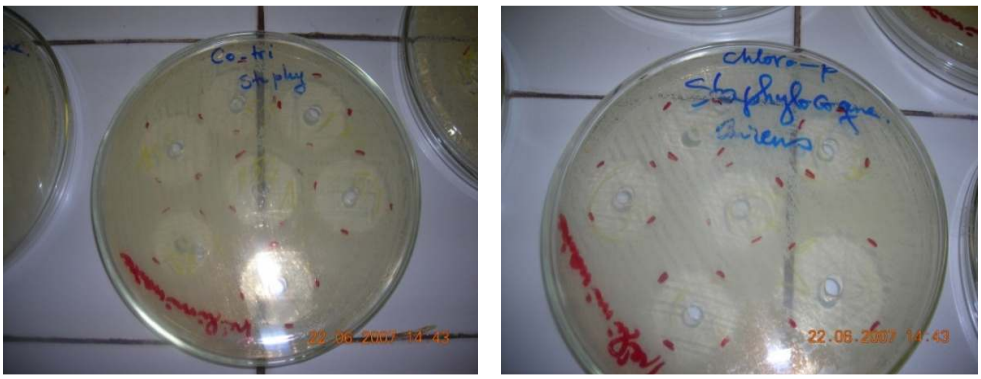

Fig. 1. Petri dish plates used and zones of inhibition

Legend: Discs contained Augmentin 30 µg; Norfloxacin 10 µg; Amoxicillin 30 µg; Ampicillin 10 µg; Chloramphenicol 30 mcg, Cotrimoxazole 25 mcg, and Erythromycin 15 mcg.

Table 1. Antibiotics tested and their commercial representative samples on Rwanda market

	Antibiotics		Dosage forms	Samples origin				
				S1	S2	S3	S4	S5
1	Ampicillin	AP	Cap250 mg	India	India	Rwanda	India	APS
2	Amoxicillin	AM	Cap500 mg	India	Italia	Italia	Rwanda	AMS
3	Augmentin	AG	Cap500/125 mg	Italia	India	India	France	AGS
4	Erythromycin	ER	Tab400 mg	India	India	Italia	India	ERS
5	Chloramphenicol	CP	Cap250 mg	India	Rwanda	Belgium	India	CPS
6	Cotrimoxazole	CT	Tab400/80 mg	India	Italia	France	Kenya	CTS
7	Norfloxacin	NF	Tab500 mg	Italia	India	Italia	India	NFS

Legend: S5 = gold standards chosen. APS(Penbriten®), AMS(Clamoxyl®), AGS(Augmentin®), ERS(Erythrocin®), CPS(Chloromycetin®), CTS(Bactrim®), NFS(Noroxin®)

2.5 Statistical Analysis

The data obtained was subjected to a statistical analysis using Window Excel and SPSS v17 statistical tools. ANOVAs tests for multiple comparisons and significant analysis ($p<0.05$) were carried out.

3. RESULTS

3.1 Concentration – Response Relationship

As shown in Table 2, there was no significant difference between prepared and commercial disc 10mcg ($p=0.097$).The linearity in inhibitory zones using 3 dose levels of Ampicillin products showed positive relationship (R^2 >0.977; y=0.35 +0.4X) and statistical difference between concentrations ($p<0.0001$).

3.2 Antimicrobial Susceptibility / Resistance

Table 3 presents the diameters of inhibition zones for the tested antibiotics. *Salmonella typhi* strain is sensitive to all NF-products, intermediate sensitive to 4 AG-products but S3, and resistant to all AP-, AM-, CT-, and ER-products. *S. flexneri* strain is sensitive to all antibiotics except ER; all ERs products showed resistance. *S. aureus* strain is sensitive to all antibiotics. Differences were comparatively significant ($p<0.001$). Fig. 2 shows the mean values of inhibitory zones calculated from five samples of each antibiotic. Norfloxacin representing fluoroquinolones is relatively active against all three bacteria strains; it is more active against *S. aureus*, intermediate against *S. flexneri* and less active against *S, typhi.* Ampicillin and Amoxicillin have no activity against *S. typhi,* but they more active against *S. flexneri* than against *S. aureus*. Augmentin is very less active against *S. typhi and* more active against *S. flexneri* than against *S. aureus.* Chloramphenicol is inactive against *S. typhi*, but more active against *S. aureus* than against *S. flexneri.* Cotrimoxazole is high active against *S. flexneri*, intermediate against *S. aureus*, but inactive against *S. typhi*. Erythromycin showed activity only against *S. aureus*.

3.3 Comparative Equivalence of Commercial Products

All antibiotic samples satisfied the qualitative tests for visual inspection as well as for chemical identification of active ingredients. Table 3 shows the relative antimicrobial equivalences determined by calculating the ratios between inhibition zones of generics and selected gold standard brands. The difference between Augm-S3 and gold standard S5 and other paired-samples was noted on *Salmonella* susceptibility (S5=100%, S1=100%, S2=92%, S3=0%, S4=92%). For *Shigella* susceptibility, Augmentin-S2 and S3 samples were less than 90% from gold standard S5, meaning that generic-S2 and S3 may be out of USP specifications (90-110%) taking S5 as reference.

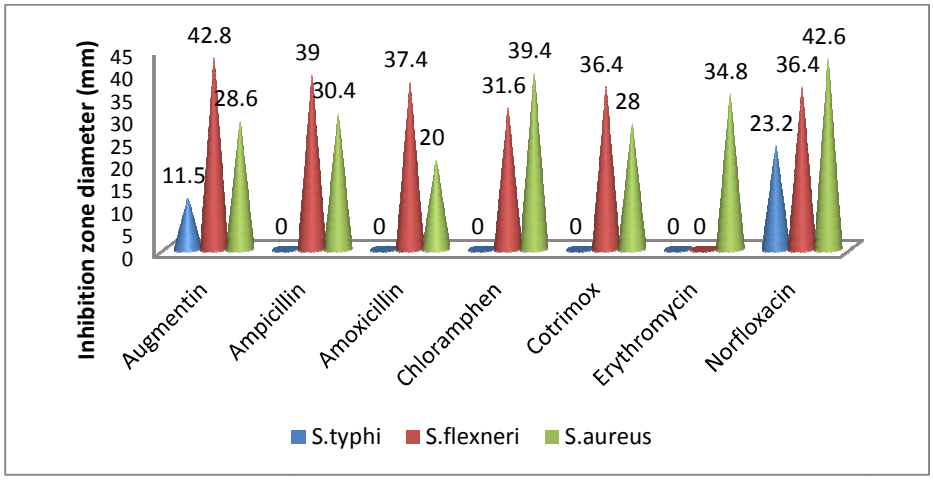

Fig. 2. Comparative susceptibility/resistance to different antibiotics, using a disc diffusion method

Legend: Salmonella typhi, Staphylococcus aureus and Shigella flexneri strains isolated from patients in Rwanda; values are means of 5 different commercial products corresponding to each antibiotic

Table 2. Inhibition zones (mm) for *S. aureus* strains as function of Ampicillin amount on discs

Samples	Prepared discs vs. Commercial disc 10 mcg			Concentration vs Inhibitory zones			
	Prepared discs	Standard disc	p-value	Prepared discs			p-value
	10 mcg	10 mcg		5 mcg	10 mcg	20 mcg	
AP1	39	39		38	39	42	
AP2	40	40		38	40	42	
AP3	39	39		36	39	43	
AP4	40	39		37	40	44	
Mean	39.50	39.25	0.097	37.25	39.50	42.75	0.000
SD	0.58	0.50		0.96	0.58	0.96	

P-value ANOVA single factor test

Table 3. Antimicrobial susceptibility/Resistance expressed in diameter of inhibition zone (mm)

Antibiotics	*Salmonella typhi*					*Shigella flexneri*					*Staphylococcus aureus*				
Samples	**S1**	**S2**	**S3**	**S4**	**S5**	**S1**	**S2**	**S3**	**S4**	**S5**	**S1**	**S2**	**S3**	**S4**	**S5**
Augmentin	12	11	0	11	12	43	45	41	43	42	30	31	27	28	27
Ampicillin	0	0	0	0	0	39	40	39	40	37	28	29	30	28	37
Amoxicillin	0	0	0	0	0	37	37	38	38	37	20	20	19	21	20
Chloramphenicol	0	0	0	0	0	31	32	32	32	31	40	40	39	40	38
Cotrimoxazole	0	0	0	0	0	36	37	37	37	35	29	27	28	28	28
Erythromycin	0	0	0	0	0	0	0	0	0	0	36	35	35	36	32
Norfloxacin	23	23	23	23	24	38	38	36	35	35	43	42	43	43	42

Each antibiotic is represented by 4 different products (S1-S4) and reference gold standard product S5.
Discs potency contained Augmentin 30 µg; Norfloxacin 10 µg; Amoxicillin 30 µg; Ampicillin 10 µg; Chloramphenicol 30 µg, Cotrimoxazole 25 µg, and Erythromycin 15 µg

Table 4. One-sample statistical comparison (Test value=0)

	T	df	Sig. (2-tailed)	Mean difference	95% confidence interval of the difference	
					Lower	Upper
S. typhi	3.211	34	.003	4.62	1.70	7.56
S. flexneri	13.863	34	.000	31.94	27.26	36.63
S. aureus	25.747	34	.000	31.97	29.45	34.50

Table 5. Comparative relative contents (%) of generic antibiotics from gold standard chosen

	Salmonella typhi					*Shigella flexneri*					*Staphylococcus aureus*				
Samples	**S1**	**S2**	**S3**	**S4**	**S5**	**S1**	**S2**	**S3**	**S4**	**S5**	**S1**	**S2**	**S3**	**S4**	**S5**
AG	100	92	0*	92	100	97	87*	87*	90	100	96	93	91	96	100
AP	-	-	-	-	-	97	103	96	97	100	98	93	98	100	100
AM	-	-	-	-	-	100	95	100	105	100	100	100	103	103	100
CP	-	-	-	-	-	100	97	95	100	100	97	97	100	100	100
CT	-	-	-	-	-	107	103	103	104	100	97	95	100	100	100
ER	-	-	-	-	-	-	-	-	-	-	103	100	91	103	100
NF	96	96	96	98	100	102	102	100	102	100	100	92	95	92	100

*Augmentin(AG), AP(Ampicillin), Amoxicillin(AM), Chloramphenicol(CP),Cotrimoxazole(CT), Erythromycin(ER), Norfloxacin(NF;*value out 90-110% range; Resistance (-)*

4. DISCUSSION

The research aimed at evaluating whether different commercial antimicrobial products corresponding to a same antibiotic were equivalent with regard to pharmaceutical quality and antimicrobial relative potency. In this study, some brands were presumptively regarded as good quality medicines to serve as gold standard for the analysis of generic preparations instead of international references that are very costly for a routine assessment. Concerning antimicrobial susceptibility/ resistance, the findings highlight some problems of resistance. We tested the activity of seven antibiotics using clinical pathogenic bacteria isolates instead of international reference strains to seek whether these common antibiotics can be used to treat patients infected with those strains.

Concerning the pharmaceutical quality, the visual inspection was satisfactory with regard to labeling and qualitative identification tests of active ingredients. The chemical quantitative assay was not performed. However the relative content in each product could be deducted from the relative ratios in inhibition zones compared to commercial brands presumptively selected as gold standards. For instance, among Augmentin-products, one sample (S3) was significantly different from the gold standard used as reference product and from other three samples for the inhibition of *Salmonella* strains. We suspected a difference in the amount of clavulanic acid since AG-S2 and AG-S3 relative potencies were less than 90-110% range required by USP. This confirms the problem of poor quality medicines to which the country faces [9].

Ampicillin and amoxicillin are penicillins that have been used to prevent and treat a number of bacterial infections including acute otitis media, streptococcal pharyngitis, pneumonia, skin infections, urinary tract infections, Lyme disease, *Chlamydia* infections, respiratory tract infections, meningitis, endocarditis and *Salmonella* infections [23,24]. The result of this study has shown that these two antibiotics are more active against *Shigella* strains than against *Staphylococcus aureus*, but are inactive against *Salmonella typhi* strains. The result is consistent with a study by others indicating that 82% and 6% of *Staphylococcus aureus* strains in Rwanda were Oxacilline- and Vancomycin-resistant respectively [14,15]. Augmentin-like preparations that associate amoxicillin and clavulanic acid showed moderate inhibition activity against *Salmonella* strains but not significantly enough to be recommended in treatment. It is known that clavulanic acid may extend the activity of amoxicillin to bacteria producing beta-lactamases. However, as it has been reported that community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) causes more than one half of all staphylococcal infections in most communities, empiric therapy with penicillins alone may be inadequate [25].

Erythromycin has been recommended to treat bacteria responsible for causing infections of the skin and upper respiratory tract, including *Streptococcus*, *Staphylococcus*, and *Haemophilus* genera. The MIC susceptibility of *Staphylococcus aureus* is from 0.023 to 1024 µg/ml [26]. The result of this study showed that Erythromycin is not active against *Salmonella* and *Shigella* strains isolated from some patients, but remains however more active against *S. aureus* than penicillins.

The original indication of chloramphenicol was in the treatment of typhoid, but the now almost universal presence of multiple drug-resistant *Salmonella typhi* has meant it is seldom used for this indication except when the organism is known to be sensitive [27]. The result of this study is consistent with this warning.

However the result showed that chloramphenicol remains active against staphylococcal infections and can be the first-choice treatment for staphylococcal brain abscesses because of its excellent blood-brain barrier penetration as compared to beta-lactams [27]. Chloramphenicol resistance may be carried on a plasmid that also codes for resistance to other drugs. One example is the ACCoT plasmid (A=Ampicillin, C=Chloramphenicol, Co=Cotrimoxazole, and T=Tetracycline), which mediates multiple-drug resistance in typhoid (also called R factors) [28].

Bactrim® was claimed to be effective in a variety of upper and lower respiratory tract infections, renal and urinary tract infections, gastrointestinal tract infections, skin and wound infections, septicemias, and other infections caused by sensitive organisms [29,30]. The result of this study has shown that Cotrimoxazole (or Bactrim) remains active against *S. aureus* and *Shigella*, but cannot be used against resistant *Salmonella* strains. Because it has a higher incidence of adverse effects, including allergic responses, its use has been restricted in many countries to very specific circumstances where its improved efficacy has been demonstrated, like in preventing opportunistic infections in HIV-infected people [31]. Resistance of *Shigella* species to sulfonamides, cotrimoxazole, tetracyclines, and ampicillin has been reported worldwide, and these agents are not recommended as empirical therapy [26].

The activity of Norfloxacin as representative of fluoroquinolones is proven against *Salmonella* strains as well as against *Shigella* and *Staphylococcal* infections [32].

5. CONCLUSIONS

These findings showed the possibility of using *in vitro* antimicrobial susceptibility testing to determine the quality and equivalence of commercial antibiotic products as well as their

spectra of activity. *Salmonella typhi* is susceptible to fluoroquinolones and no more to chloramphenicol. *Shigella flexneri* is susceptible to six tested antibiotics except Erythromycin. *Staphylococcus aureus* is susceptible to all seven tested antibiotics. The result will help clinicians choosing the most appropriate antibiotic product and the health authority issuing appropriate recommendations.

CONSENT

It is not applicable.

ETHICAL ISSUE

The protocol was approved by the Research Ethical Committee and Medical Ethical committee of the hospital.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Centers for disease control and prevention, food and drug administration, national institutes of health. A Public Health Action Plan to Combat Antimicrobial Resistance. Available:http://www.cdc.gov/drugresistance/pdf/2010/Interagency-Action-Plan-PreClearance-03-2011.pdf
2. World Health Organization. WHO report 2012: The evolving threat of antimicrobial resistance: Options for action (Executive Summary). 2012;6–14.
3. World Health Organization. WHO report 2013: Antimicrobial Drug Resistance, Geneva. 2013;1–5. Available:http://apps.who.int/gb/ebwha/pdf_files/EB134/B134_37-en.pdf
4. World Health Organization. WHO Report. Antimicrobial Resistance: Global Report on Surveillance (Summary). 2014;3–6.
5. Shears P. Antibiotic resistance in the tropics: Epidemiology and surveillance of antimicrobial resistance in the tropics. Trans R Soc Trop Med Hyg. 2001;95:127–30.
6. WHO. Counterfeit Drugs Guidelines; 1999. Available:http://apps.who.int/medicinedocs/pdf/h1456e/h1456e.pdf
7. WHO. Fact sheet revised, essential medicines and health products—Counterfeit Medicines; 2006. Available:www.who.int/medicines/services/counterfeit/impact/ImpactF_S/en/
8. Kelesidis T, Kelesidis L, Rafailidis PI, and Falagas ME. Counterfeit or substandard antimicrobial drugs: A review of the scientific evidence. Journal of Antimicrobial Chemotherapy. 2007;60(2):214-236.
9. Habyalimana V, Mbinze JM, Kalenda NT, Dispas A, Loconon AY, Sacré PY, Widart J, De Tullio P, Counerotte S, Kadima NJL, Ziemons E, Hubert P, Marini RD. Analytical tools and strategic approach to detect poor quality medicines, identify unknown components, and timely alerts for appropriate measures: Case study of antimalarial medicines. Am J of Analytical Chemistry. 2015;6:977-994. Available:http://dx.doi.org/10.4236/ajac.2015.613093
10. Okeke I, Aboderin O, Byarugaba D, Ojo K, Opintan J. Growing problem of multidrug-resistant enteric pathogens in Africa. Emerg Infect Dis. 2007;13:1640–5.
11. Omulo S, Thumbi SM, Njenga MK, and Call DR. A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: what can be done better? Antimicrobial Resistance and Infection Control. 2015;4:1. DOI: 10.1186/s13756-014-0041-4
12. Petat E, Carteron B, Reguer M, Lemmens P, Vandepitte J, Ghysels G. *Shigella* and *Salmonella* isolated in Burundi from 1980 to 1985. Bull Soc Pathol Exot Filiales. 1987;80:171–9.
13. Ndihokubwayo JB, Baribwira C, Ndayiragije A, Poste B. Antibiotic sensitivity of 299 strains of *Shigella* isolated in Burundi. Med Trop. 1996; 56:37–40.
14. Ntirenganya C, Manzi O, Muvunyi CM, Ogbuagu O. High prevalence of antimicrobial resistance among common bacterial isolates in a tertiary healthcare facility in Rwanda. Am J Trop Med Hyg. 2015; 92(4):865-70. DOI: 10.4269/ajtmh.14-0607
15. Bogaerts J, Verhaegen J, Munyabikali JP, Mukantabana B, Lemmens P, Vandeven J, et al. Antimicrobial resistance and serotypes of *Shigella* isolates in Kigali, Rwanda (1983 to 1993): Increasing frequency of multiple-resistance. Diagnostic Microbiol Infect Dis. 1997;28: 165–71.
16. Lepage P, Bogaerts J, van Goethem C, Hitimana D, Nsengumuremyi F.

Multiresistant Salmonella typhimurium systemic infection in Rwanda. Clinical features and treatment with cefotaxime. J Antimicrob Chemother. 1990;26(Suppl A): 53–7.

17. Vimont-Vicary P, Rogerie F. 3-year study of shigellosis epidemic in Rwanda, Central Africa. Problems of public health and bacteriological aspects. Med Trop. 1985;45:235–43.
18. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial susceptibility Testing; Twenty-first informational supplement. M100-S21. 2011;31(1).
19. Clinical Bacteriology: Basic Technique for WHO laboratory, Second Ed, Geneva; 1994.
Available:whqlibdoc.who.int/.../2003/9241545453.pdf
20. Zuluaga AF, Agudelo M, Rodriguez CA, Vesga O. Application of microbiological assay to determine pharmaceutical equivalence of generic intravenous antibiotics. BMC Clinical Pharmacology. 2009;9:1.
21. Jones RN, Fritsche TR, Moet GJ. In vitro potency evaluations of various piperacillin/ tazobactam generic products compared with the contemporary branded (Zosyn®, Wyeth) formulation. Diagnostic Micro-biology and Infectious Disease. 2008; 61:76–79.
DOI: 10.1016/j.diagmicrobio.2007.12.010
22. Jorge A, Diaz JA, Silva E, Arias MJ, and Garzón M. Comparative *in vitro* study of the antimicrobial activities of different commercial antibiotic products of vancomycine. BMC Clin Pharmacol. 2011;11:9.
23. "Ampicillin". In Bertram G. Katzung-Basic & Clinical Pharmacology (8th Edition), pp753-813.
24. "Amoxicillin". In Bertram G. Katzung-Basic & Clinical Pharmacology (8th Edition), pp 753-813.
25. Shiferaw B, Solghan S, Palmer A, Joyce K, Barzilay EJ, Krueger A, Cieslak P. Antimicrobial susceptibility patterns of *Shigella* isolates in Foodborne Diseases Active Surveillance Network (FoodNet) sites, 2000-2010. Clin Infect Dis. 2012; 54(Suppl 5):S458-63.
DOI: 10.1093/cid/cis230
26. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. Clin Infect Dis. 2002;34(4): 482-492.
DOI: 10.1086/324626
27. "Chloramphenicol". In Bertram G. Katzung-Basic & Clinical Pharmacology (8th Edition), pp 735-813.
28. Chloramphenicol resistance. Available:http://www.liquisearch.com/chloramphenicol/resistance
29. Mandal S, DebMandal M, Pal NK. Antibiotic resistance of Salmonella enteric Serovar Typhi in Kolkata, India, and *In Vitro* experiments on effect of combined chemotherapy. The Scientific World Journal. 2012;4. Article ID 454059.
DOI: 10.1100/2012/454059
30. "Co-trimoxazole" In Bertram G. Katzung-Basic & Clinical Pharmacology (8th Edition), pp 735-813.
31. Mermin J, Lule J, Ekwaru JP, Malamba S, Downing R, Ransom R, Kaharuza F, Culver D, Kizito F, Bunnell R, Kigozi A, Nakanjako D, Wafula W, Quick R. Effect of co-trimoxazole prophylaxis on morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda. The Lancet. 2004;364(9443):1428–1434.
DOI: 10.1016/S0140-6736(04)17225-5
32. Padeĭskaia EN. Norfloxacin: More than 20 years of clinical use, the results and place among fluoroquinolones in modern chemotherapy for infections. Antibiot Khimioter. 2003;48(9):28–36.

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