Scholars Academic Journal of Pharmacy (SAJP)
Abbreviated Key Title: Sch. Acad. J. Pharm.
©Scholars Academic and Scientific Publisher
A Unit of Scholars Academic and Scientific Society, India
www.saspublisher.com

ISSN 2347-9531 (Print)
ISSN 2320-4206 (Online)

Medicine

Evaluation of Antibacterial Activity and Synergistic Effect of Different Conventional Antibiotics against Diarrheal Bacteria

Joseph Privat Ondo[1-2]*, Guy Stéphane Padzys[1-3], Cédric Sima Obiang[1], Guy-Roger Ndong Atome[1-2], Morel Essono Mintsa[1], Rebecka Emmanuelle Moungoyi Massala[1], Louis-Clément Obame Engonga[1-2], Joel-Fleury Djoba Siawaya[4]

[1]Laboratoire de Recherche en Biochimie (LAREBIO), Université des Sciences et Techniques de Masuku B.P. 769, Franceville, Gabon.
[2]Laboratoires de Substances Naturelles et de Synthèses Organométalliques (LASNSOM), Université des Sciences et Techniques de Masuku B.P. 943, Franceville, Gabon.
[3]Département de Biologie Université des Sciences et Techniques de Masuku BP: 943, Franceville, Gabon.
[4]Service d'Endocrinologie Centre Hospitalier Universitaire de Libreville, BP 2228, Libreville, Gabon

Original Research Article

***Corresponding author**
Joseph Privat Ondo

Article History
Received: 23.12.2017
Accepted: 08.01.2018
Published:30.01.2018

DOI:
10.21276/sajp.2018.7.1.2

Abstract: The present study has been carried out to evaluate the activity of antibiotics alone and antimicrobial potential of their combination against bacteria implicated in infectious diseases like diarrhoea in tropical areas. The antimicrobial potential of antibiotics was tested against clinical isolates and reference bacterial strains using disk diffusion and microdilution methods. The results showed that the combined application of the antibiotics led to a synergistic effect in some cases, but antagonistic effect was also observed in some bacteria. Combinations involving CIP together with another antibiotic were the most efficient. Combinations CIP+MTZ and AMX+CIP were synergistic and have bactericidal activity against *S. aureus*, *P. aeruginosa*, respectively. While the synergistic combination of CIP+AMP was bactericidal against *S. aureus* and *P. aeruginosa* (MBC / MIC = 1). The use of the antibiotic combination depends on the antibiotics used and the infectious bacterial strain. The choice of antibiotic combinations should be dictated by results of susceptibility tests performed on each strain.

Keywords: Antibiotic combination; bacteria diarrhoeic; multidrug resistance; synergistic effect; antimicrobial activity; antagonistic effect.

INTRODUCTION

Enteric diseases are a major cause of morbidity and mortality in poor and developing countries [1]. Diarrhea is the clinical syndromes of digestive expression of bacterial, parasitic or viral origin, linked to the fecal peril.

They are a major health problem in the world, especially in Third World countries where these diseases are endemic. According to WHO, the number of infant diarrhea deaths is estimated to 5-10 million per year. In developing countries, nearly 760,000 children under five die from diarrhea [2]. The causative organisms are Gram-negative bacteria including Enterobacteriaceae (*E. coli*, *Shigella* and *Salmonella*); and Gram-positive bacteria (staphylococci); they developed the mechanisms of resistance to antibiotics likely to compensate for diarrhea [3].

Indeed, multiple modalities including antibiotic therapies have been used to treat these common infections. But recently, this has been put in jeopardy by emergence of widespread antimicrobial resistance, which is one of the major problems facing modern medicine. Thus, innovative therapeutic methods to combat antibiotic-resistant bacterial pathogens are urgently required.

There are many ideas and strategies about the possibility of keeping control of infections in this "antibiotic resistance crisis" current [4, 5]. One of them consists in monitoring the effectiveness of drugs already on the market [6], like that, inspect the enhancement of antibiotic efficacy by their combination. Combining antibiotics is a promising strategy to increase treatment efficacy and to control resistance evolution [7].

Currently the most used antibiotics in Gabon against diarrhea are ciprofloxacin, metronidazole, amoxicillin and ampicillin. It is therefore necessary to control the effectiveness of these antibiotics with regard to the main germs causing diarrhea and to seek

therapeutic solutions, in particular by combining antibiotics to combat resistant organisms.

MATERIALS AND METHODS

Microbial strains

The selection of clinical microorganisms depended on their availability, thus microorganisms that have been reported to be the most frequently implicated in infectious diseases like diarrhoea in tropical areas were well-represented [8]. Some antibiotics and their combinations were tested against a panel of resistant microorganisms, including five strains non-referenced of clinical isolates obtained from diarrhea affected patients (different ages); *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae spp pneumonia*, *Pseudomonas aeruginosa*, *Shigella spp* why were isolated at Laboratory of Bacteriology of University Hospital Center of Libreville, Gabon. Five references bacterial strains, *Escherichia coli* CIP 105182, *Salmonella typhimurium* ATCC 13311, *Shigella dysenteria* CIP 5451, *Salmonella entérica* CIP 105150, *Staphylococcus aureus* ATCC 25293 BHI were tested too.

Antibiotics used in this study and Preparation

Antibiotic powders of ampicillin (AMP), amoxicillin (AMX, BAILLY-CREAT, France), metronidazole (MTZ, SANOFI AVENTIS, France) and ciprofloxacin (CIP, EXPHAR, Belgique) were used. The combinations of antibiotics used are Ampicilin + Metronidazole (AMP+MTZ); Amoxicillin + Ciprofloxacin (AMX+CIP); Amoxicillin + Ampicilin (AMX+AMP); Amoxicillin + Metronidazole (AMX + MTZ); Ciprofloxacin + Metronidazole (CIP+MTZ); Ciprofloxacin + Ampicilin (CIP+AMP).

For antibacterial screening, stock antibiotic solutions were prepared (100 mg/mL) for each and combinations. In addition, different concentrations of antibiotic combinations, which is to be examined for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), are prepared (2.5 - $7.62.10^{-5}$ mg/mL).

Antibacterial screening of antibiotics

The antibacterial screening of antibiotics alone and combination was carried out using by agar well diffusion method [9]. The bacteria grown in nutrient broth at 37°C for 18 h were standardized using normal saline to turbidity of 0.5 Mac Farland standards (10^8 cfu/mL). Petri dishes (90 mm in diameter) were prepared with 15 mL of a base layer of Müeller-Hinton gelose medium and the test bacteria were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Six millimeter of sterile paper discs (Whatman No. 3) soaked with 20 µL of the antibiotics dilution or combinations (100 mg/mL) were placed on agar in 15 mm of Petri dishes periphery. The Petri dishes were incubated aerobically at 37°C for 18 to 24 h. The effect of antibiotic and combination was reflected by the appearance around disc with a transparent circular zone corresponding to the absence of growth. The diameter of inhibition zone was measured in mm. All tests were performed in triplicate and antibacterial activity was expressed as the mean of Diameters of Inhibition Zone (DIZ) produced.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimal Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were studied for the bacterial strains and the antibiotic combinations that presented of synergetic action in disc diffusion assay. Thus, a microdilution broth susceptibility assay was used, as recommended by the National Committee for Clinical Laboratory Standards [9] for determination of MIC and MBC. Briefly, the combinations were properly prepared, sterilized and 100 µL (10 mg/mL) transferred to first row of wells in sterile 96 well-plates previously filled with 100 µL of sterile nutrient broth to obtain a twofold serial dilutions. Then, 90 µL of nutrient broth and 10 µL of microbial suspensions diluted from the same 0.5 Mac Farland standards to have 5 x 10^5 CFU/mL in each well were added into wells. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no antibiotic added). A range of concentration of combination from 2.5 to $7.62.10^{-5}$ mg/mL was prepared in a total volume in wells of 200 µL. The plates were incubated aerobically at 37°C and MICs were determined. The MIC was defined as the lowest concentration of the combination at which the microorganism tested does not demonstrate visible growth.

To determine MBCs, 100 µL of bacterial suspension from subculture demonstrating no visible growth were removed to spread onto Plate Count Agar (PCA) medium plates. Plates were incubated at 37°C for a total period of 48h. The MBC was defined as the lowest concentration of the combination at which 99.99% or more of initial inoculum was killed.

Statistical Analysis

Experimental results were expressed as mean ± standard deviation. All measurements were replicated three times.

RESULTS

Diameter of inhibition zone of antibiotics and combination

The antibacterial activity of the antibiotics as a function of the diameter of the zone of inhibition (DZI) is interpreted as follows: DZI < 8 mm: non-sensitive; 8 mm ≤ DZI ≤ 14 mm: sensitive; 15 mm≤ DZI ≤ 19 mm: very sensitive; DZI ≥20 mm: extremely sensitive [10].

Antibiotics alone

In table 1, the antibiotic resistance/ susceptibility profile of reference strains revealed that,

the antibiotics showed significant antibacterial characters against the tested microorganisms referenced with exception of *Staphylococcus aureus* ATCC 25293 BHI that has resistance to metronidazole.

While, the analysis in table 2 reveals that the clinical isolates are all highly sensitive to at least one of the four antibiotics tested. Nevertheless, several isolates are resistant to at least one antibiotic. Thus, *Staphylococcus aureus* is resistant to AMX and CIP whereas *Klebsiella pneumoniae spp pneumoniae* and *Pseudomonas aeruginosa* are resistant to MTZ and CIP, respectively. Shigella spp has a multi-resistance against AMP, MTZ and AMX antibiotics.

Antibiotic combinations

The reference strains exhibit a different sensitivity to combinations of antibiotics than clinical isolates. According to the obtained results (table 1), the combination of the antibiotics showed that most of the reference strains were an antagonistic effect to one or more of the tested combination. Most of the strains were an antagonistic effect to AMX+AMP combination (100%), AMX+CIP and AMX+MTZ combinations (80% respectively), CIP+MTZ and CIP+AMP combinations (60% respectively).

Four combinations of antibiotics have an activity, which is indifferent to at least one reference strain. Indeed, the AMP+MTZ and CIP+AMP combinations have an indifferent activity on two strains, *Shigella dysenteria* CIP 5451 and *Staphylococcus aureus* ATCC 25293 BHI as well as *Escherichia coli* CIP 105182 and *Salmonella enterica* CIP 105150 respectively. The combinations AMX+CIP, AMX+MTZ have indifferent action to *Escherichia coli* CIP 105182. The application of AMP with MTZ led to a synergistic effect on *Salmonella enterica* CIP 105150 and *Salmonella typhimurium* ATCC 13311. A synergistic effect was also observed in *Salmonella enterica* CIP 105150 and *Staphylococcus aureus* ATCC 25293 BHI when the combination of CIP with MTZ was applied.

With regard to clinical isolates, table 2 shows that they have less antagonistic activity against the combinations compared to the reference strains. Thus, the combination of AMP+MTZ antibiotics shows 80% of antagonistic action, AMX+AMP has 60%, CIP+MTZ and CIP+AMP, and AMX+CIP and AMX+MTZ have 40% and 20% antagonistic effect, respectively. The antibiotic combinations studied have more synergistic activity on clinical isolates. Indeed, five of six combinations have a synergistic action on at least two isolates. Thus, the AMX+MTZ combination is the most effective on isolates because it has a synergistic effect on four (4) isolates including, *Staphylococcus aureus*, *Klebsiella pneumoniae spp pneumoniae*, *Pseudomonas aeruginosa*, *Shigella spp*. The combinations AMX+CIP, CIP+MTZ and CIP+AMP all have a synergistic effect with *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates. A synergistic action was also observed in *Escherichia coli* and *Shigella spp* when the combination of AMX+AMP was applied. An indifferent activity of the combination of the antibiotic AMP+MTZ on *Shigella spp* isolate, AMX+CIP was observed on *Escherichia coli* and *Klebsiella pneumoniae spp pneumonia* isolate. Finally, CIP+MTZ and CIP+AMP have an indifferent effect on *Klebsiella pneumoniae spp pneumoniae*.

Table-1: The antimicrobial activities (zones of inhibition) of antibiotics alone and its combination effect (mm) against five strains referenced

Microorganisms	Standard antibiotic				Antibiotic combinations					
	AMP	MTZ	AMX	CIP	AMP+MTZ	AMX+CIP	AMX+AMP	CIP+MTZ	CIP+AMP	AMX+MTZ
Escherichia coli CIP 105182	12 ± 0.66	20 ± 2.0	19.33 ± 0.44	38.66 ± 1.11	16 ± 1.11 A	38 ± 2.0 I	R, A	35.33 ± 0.44 A	37.33 ± 1.55 I	19.66 ± 0.44 I
Shigella dysenteria CIP 5451	14.33 ± 1.11	13 ± 2.0	16.66 ± 1.11	40.66 ± 0.88	14.33 ± 1.11 I	R, A	10.66 ± 0.88 A	35.33 ± 0.44 A	37.66 ± 1.77 A	10 ± 1.33 A
Salmonella enterica CIP 105150	36.33 ± 1.11	36.33 ± 4.22	27 ± 1.33	38 ± 2.0	55 ± 6.66 S	R, A	28.33 ± 2.22 A	41 ± 1.33 S	38.66 ± 1.11 I	17.33 ± 2.22 A
Salmonella typhimurium ATCC 13311	35 ± 0.66	31.66 ± 1.55	35 ± 0	48.66 ± 2.44	41 ± 0.66 S	R, A	16.66 ± 2.22 A	41.66 ± 2.22 A	35.66 ± 3.77 A	14.33 ± 1.55 A
Staphylococcus aureus ATCC 25293 BHI	43.33 ± 1.77	R	36 ± 0.66	21.66 ± 2.2	43.33 ± 1.77 I	R, A	38.33 ± 2.22 A	30 ± 0 S	39.66 ± 0.44 A	16 ± 0.66 A

I: Indifference; S: Synergy; A: Antagonism; R: resistant strain (no inhibition)

Table-2: The antimicrobial activities (zones of inhibition) of antibiotics alone and its combination effect (mm) against five resistant clinical isolates

Microorganisms	Standard antibiotic				Antibiotic combinations					
	AMP	MTZ	AMX	CIP	AMP+MTZ	AMX+CIP	AMX+AMP	CIP+MTZ	CIP+AMP	AMX+MTZ
Escherichia coli	28.5 ± 2.0	45 ± 0.1	34 ± 2.1	55 ± 0.1	39.33 ± 0.89 A	53.33 ± 2.22 I	43.33 ± 2.22 S	50 ± 3.3 A	41.67 ± 2.22 A	41.67 ± 2.22 A
Staphylococcus aureus	34 ± 1.0	25 ± 2.2	R	R	27 ± 0.67 A	45 ± 0.1 S	29.33 ± 2.89 A	43.33 ± 2.22 S	43.33 ± 2.22 S	31.33 ± 2.5 S
Klebsiella pneumoniae spp pneumoniae	18.5 ± 0.5	R	11.5 ± 1.5	28.5 ± 1.5	R, A	30 ± 0.1 I	R, A	29.33 ± 0.89 I	27.33 ± 0.44 I	16 ± 0.67 S
Pseudomonas aeruginosa	30.5 ± 1.2	20 ± 0.1	22.5 ± 2.2	R	26 ± 2.3 A	47.67 ± 1.78 S	28 ± 0.1 A	51 ± 3.1 S	48.67 ± 0.89 S	43.33 ± 2.22 S
Shigella spp	R	R	R	29 ± 1.0	R, I	20 ± 0.1 A	41.67 ± 2.22 S	27 ± 1.33 A	24.33 ± 0.89 A	41.67 ± 2.22 S

I: Indifference; S: Synergy; A: Antagonism, R: resistant clinical isolate (no inhibition)

MICs and MBCs of the antibiotic combinations

The MIC and MBC values, those resulting from combining two antibiotics with synergetic action in disc diffusion assay, are presented in table 3. The results of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of antibiotic combination against different microorganisms shows that CIP+MTZ combination had a wider spectrum of activity than others. It had MIC of 0.04 mg/mL against *S. aureus*, *P. aeruginosa* clinical isolates and $7.62.10^{-5}$ mg/mL, $6.1.10^{-4}$ mg/mL against *S. enterica* CIP 105150 and *S. aureus* ATCC 25293 BHI, respectively. It also had minimum bacterial concentration of 0.04 mg/mL against *S. aureus*, 0.08 mg/mL against *P. aeruginosa*, and $6.1.10^{-4}$ mg/mL against *S. enterica* CIP 105150, $4.9.10^{-3}$ mg/mL against *S. aureus* ATCC 25293 BHI.

The CIP+AMP combination also showed good activity against *S. aureus* and *P. aeruginosa* isolates with 0.01 mg/mL MIC and 0.08 mg/mL MBC on each of the isolates, respectively. The same is true for the combination of antibiotic AMX+CIP with MIC = 0.04; 0.62 mg/mL and MBC = 0.16; 0.62 mg/ml for *S. aureus* and *P. aeruginosa*, respectively. The AMX+MTZ combination showed no inhibitory activity against *P. aeruginosa* and *Shigella spp* in the concentration range of our study. The same result was found for AMX+AMP combination against *Shigella spp* and *E. coli*; its shows a MIC of 1.25 mg/mL and an MBC

superior than 2.5 mg/mL. AMP+MTZ shows an interesting result on *S. enterica* CIP 105150 and *S. typhimurium* ATCC 13311 for MIC (0.32 and 0.62 mg / mL, respectively) but the MBC is greater than 2.5 mg / mL on the two strains.

Table-3: MIC and MBC values of synergistic antibiotic combinations

Microorganisms	MIC/MBC (mg/mL)											
	AMP+MTZ		CIP+MTZ		AMX+AMP		AMX+MTZ		AMX+CIP		CIP+AMP	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Clinical isolates												
E. coli					1.25	≥ 2.5						
S. aureus			0.04	0.04					0.04	0.16	0.04	0.04
P. aeruginosa			0.04	0.08			Nd	Nd	0.62	0.62	0.08	0.08
Shigella spp					Nd	Nd	Nd	Nd				
Reference strains												
S. enterica CIP 105150	0.32	≥ 2.5	$7.62.10^{-5}$	$6.1.10^{-4}$								
S. typhimurium ATCC 13311	0.62	≥ 2.5										
S. aureus ATCC 25293 BHI			$6.1.10^{-4}$	$4.9.10^{-3}$								

Nd: no determined (no inhibition)

DISCUSSION

From this study, we can see that all antibiotics showed antibacterial activity against different bacterial strains and isolates, but at different levels. All the tested bacteria were more or less sensitive to four antibiotics with the exception *S. aureus* ATCC 25293 BHI which showed resistance against MTZ. There is more resistance in clinical isolates against antibiotics, *Staphylococcus aureus* (resistant to AMX and CIP), *Klebsiella pneumoniae spp pneumoniae* (resistant to MTZ), *Pseudomonas aeruginosa* (CIP resistant), *Shigella spp* (resistant to AMP, MTZ and AMX).

In our study, we found CIP and AMX to be the most effective antibiotics against our reference strains. On the other hand, two clinical isolates were resistant to CIP (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) whereas he was highly effective against *Escherichia coli*, *Klebsiella pneumoniae spp pneumonia*, *Shigella spp*. A single isolate is resistant to AMP (*Shigella spp*) and the others are at least very sensitive. This information may be useful in the treatment of bacterial diseases such as diarrhea. AMP is therefore more suited for antibacterial therapy because, despite the greater antibacterial efficacy of CIP, it selects more resistant isolates than AMP. Although CIP is a broad spectrum antibiotic, Syeda et *al*., showed that ciprofloxacin is 21.95% and 44.44% resistant to *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. But too 27.02%, 16.66% and 72.22% resistant to *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia*, respectively [11].

Due to the frequent resistance development during monotherapy treatment of infected patients, multiple combinations of antibacterial agents are being proposed. Indeed, the clinical use of antimicrobials combinations justified to the prevention of emergence of resistant organisms, polymicrobial infection, initial therapy (in patients where the nature of infection is not clear yet) and decreased toxicity [12]. This study indicates that some combinations of standard antibiotics studied have significant potential for the development of new antimicrobial treatment and reduction of drug resistance, which will permit to find the treatment of several diseases caused by microorganisms. Indeed, from the results obtained, two combinations have a synergistic effect on three reference strains. Whereas five clinical isolates tested are individually sensitive by a synergistic effect to at least one of six microorganisms. This synergy could lead to new options for the treatment of infectious diseases. This is to effectively treat mixed and severe infections, enhance antibacterial activity, reduce the time needed for long-term antimicrobial therapy and prevent the emergence of resistant microorganisms [13].

The combinations are used in order to enhance the effect of individual antimicrobials by means of synergic interactions [14]. However, even with those pairs where one or both antibiotics exerted a bactericidal action, synergism did not occur. Indeed, some mixtures, which were strongly synergistic when acting on some strains, gave only indifference or even antagonistic effect on other strains. CIP+MTZ or AMX+CIP were synergistic against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in broth but not against *Escherichia coli*, *Shigella spp* (antagonistic effect) and *Klebsiella pneumoniae spp pneumonia* (indifference effect), in spite of the ability of certain drugs to kill a few organisms severally. In a prospective study of 189 consecutive episodes of *P. aeruginosa* bacteremia, the investigators found that survival was no greater in patients who received two or more antibiotics

with *in vitro* activity against *P. aeruginosa* (therapies not specified) than in patients who received a single agent with *in vitro* activity [13]. It should be noted that the bacteria tested were at least sensitive to the combinations studied despite the antagonistic activities observed. Only the combination AMX+CIP exhibits an antagonistic antibacterial activity leading to resistance of the reference strains (*S. dysenteria* CIP 5451, *S. enterica* CIP 105150, *S. typhimurium* ATCC 13311, *S. aureus* ATCC 25293 BHI). The clinical isolate *Klebsiella pneumoniae spp. Pneumonia* was resistant to AMP+MTZ and AMX + AMP due to an antagonistic effect of these combinations.

Clinicians will consider antibiotic therapy against resistant bacteria. The antibacterial activity was considered bactericide when the ratio MBC/MIC is 1 and bacteriostatic when this ratio is 2 or more [15]. Three combinations containing the CIP have very low MICs, marking their bactericidal and bacteriostatic effect. The three combinations were synergistic with bactericidal effect against *S. aureus*, *P. aeruginosa* isolates, *S. enterica* CIP 105150, *S. aureus* ATCC 25293 BHI strains in this study. Combinations CIP+MTZ and AMX+CIP were synergistic and have bactericidal activity against *S. aureus*, *P. aeruginosa*, respectively. While the synergistic combination of CIP+AMP was bactericidal against *S. aureus* and *P. aeruginosa* (MBC / MIC = 1). These combinations can help in combating the resistance of these bacteria to antibiotics at low concentrations. The AMX+CIP combination was bacteriostatic on *S. aureus* whereas CIP+MTZ was as bacteriostatic on the *P. aeruginosa* isolate as on *S. enterica* CIP 105150 and *S. aureus* ATCC 25293 BHI (MBC / MIC ≥ 2). Combinations of AMX+AMP and AMX+MTZ, despite their synergistic effect, require high concentrations (< 2.5 mg / mL) to prevent bacterial growth.

CONCLUSION

The practice of multidrug therapy worldwide and some *in vitro* antimicrobial combinations studies have been undertaken to validate the role of synergism in antibiotherapy. Nevertheless, our study shows that the combination of antibiotics is not recommended for all situations of resistance to antibiotics. The use of the antibiotic combination depends on the antibiotics used and the infectious bacterial strain. In some strains and isolates, combination antimicrobial therapy may be superior to monotherapy for the treatment of infections, or be useless, or even less effective than monotherapy. Hence, this provides an effective alternate way to deal with the problem of multidrug resistance if and only if the therapeutic combinations are used after an appropriate antibiogram.

ACKNOWLEDGEMENTS

The authors are very much thankful to the Shell Gabon for the financial support of materials in Laboratoire de Recherche en Biochimie (LAREBIO) USTM. They appreciate the assistance and participation of Dr. Joanna OMBOUMA especially for English corrections. We thank the Laboratory of Bacteriology of University Hospital Center of Libreville staff for assistance in technical aspects and data collection, respectively.

DISCLOSURE

The authors declare that there are no competing interests. All the authors read and approved the final version.

REFERENCES

1. Kumari S, Ambasta SK. Isolation of Microorganisms from Stool Sample of Diarrhea patient and effect of antibiotics and Herbal extract. J. Microbiol.;2(2):24-32.
2. World Health organization (WHO). Maladies diarréhiques. Aide-mémoire N° 330. 2013; Available at: www.who.int/mediacentre/factsheets/fs330/fr. Accessed April 16, 2016.
3. Ebongue CO, Tsiazok MD, Mefo'o JP, Ngaba GP, Beyiha G, Adiogo D. Evolution de la résistance aux antibiotiques des entérobactéries isolées à l'Hôpital Général de Douala de 2005 à 2012. The Pan African Medical Journal. 2015;20.
4. Spellberg B, Bartlett J, Gilbert DN. The future of antibiotics and resistance. N Engl J Med. 2013; 368:299–302.
5. Nathan C and Cars O. Antibiotic resistance–problems progress and prospects. N Engl J Med. 2014; 371:1761–63.
6. World Health Organization (WHO). 2015. Worldwide country situation analysis: response to antimicrobial resistance. Available at:
1. http://www.who.int/drugresistance/documents/situationanalysis/en/. Accessed April 14, 2016.
7. Bollenbach T. Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. Curr Opin Microbiol. 2015; 27:1–9.
8. Bonfiglio G, Simporé J, Pignatelli S, Musumeci S, Solinas ML. Epidemiology of bacterial resistance in gastro-intestinal pathogens in a tropical area. Int J Antimicrob Agents. 2002; 20:387-389.
9. National Committee for Clinical Laboratory Standards. Methods for Dilution, Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 5th Edn., Vol. 17, Approved Standards-M7-A4. NCCLS Document M7-A7, USA. 2006.
10. Ponce AG, Fritz R, Del Valle C, Roura SI. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensm. Wiss. Technol. 2003; 36:679-684.
11. Syeda QA, Ale Z, Baquar SN, Shahjahan S, Rabia B. Resistance pattern of ciprofloxacin against different pathogens. Oman Med. J. 2010; 25(4):294-298.

12. Olajuyigbe OO and Afolayan AJ. *In vitro* synergy and time-kill assessment of interaction between kanamycin and metronidazole against resistant bacteria. Afr. J. Pharm. Pharmacol. 2013; 7(18): 1103-1109.
13. Pranita DT, Sara EC, Lisa LM. Combination Therapy for Treatment of Infections with Gram-Negative Bacteria. Clin. Microbiol. Rev. 2012; 25(3): 450–470.
14. Monzón M, Oteiza C, Leiva J, Amorena B. Synergy of different antibiotic combinations in biofilms of Staphylococcus epidermidis. J. Antimicrob. Chemo. 2001; 48: 793-801.
15. Zongo C, Akomo EF, Savadogo A, Obame LC, Koudou J, Traore AS. In vitro antibacterial properties of total alkaloids extract from Mitragyna Inermis (Willd.) O. Kuntze, a west African traditional medicinal plant. Asian Journal of Plant Sciences. 2009;8(2):172-7.