World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.008

QUALITY ASSESSMENT OF SOME ANTACID SUSPENSIONS MARKETED IN PORT HARCOURT METROPOLIS, SOUTH-SOUTH NIGERIA

Chidozie N. E. Ibezim, Onyeka C. Chukwuekem, Hanson I. Ogbu*

Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, University Park, University of Port Harcourt, Nigeria.

***Corresponding Author: Hanson I. Ogbu**
Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, University Park, University of Port Harcourt, Nigeria.

Article Received on 19/09/2018 **Article Revised on 10/10/2018** **Article Accepted on 31/10/2018**

ABSTRACT

Sequel to health hazards and overall product spoilage, microbiologically related non-sterile products recalls abound. Thus, Pharmaceutical products must satisfy standard microbiological quality test before they are considered fit for their intended use. The aim of this study was to evaluate pharmaceutical and microbiological qualities as well as the prevalence and resistance patterns of objectionable microorganisms in antacid suspensions commonly sold in retail outlets in Port Harcourt Metropolis. Thirteen (13) brands of antacid suspensions were subjected to pharmaceutical and microbiological examinations using standard conventional techniques. The pH values of all the samples analyzed were in the alkaline range (7.43 – 9.45), while the sedimentation volume values ranged from 0.54 – 0.96. Though samples screened were uncontaminated with fungi however, objectionable organisms *Escherichia coli* (30.77 %) and *Klebsiella pneumoniae* (7.69 %) were predominant. The individual organism exhibited different patterns of resistance across the antibiotics tested. The study emphasizes the need to maintain good standards by adhering to Good Manufacturing Practice as a way of eliminating undesirable microorganism from products.

KEYWORDS: Assessment, Antacids, Contamination, Objectionable, Antibiotics, Susceptibility.

INTRODUCTION

At the beginning of the 21st century, microbial contamination of non-sterile products became one of the major reasons for product recalls and production shutdowns.[1] This is no doubt because the presence of certain microorganisms in non-sterile pharmaceutical products adversely affects the therapeutic activity of the product and can put the health of the patient at risk.[2] As the microbiological quality of these products reaches the threshold, routine assessment of bacteria and fungi populating within these drugs is required to ensure consumer safety.[3] Antacids, a non-sterile formulations in suspension or tablet dosage forms, are a group of medicines used for rapid relief of mild or sporadic symptoms.[4] Their primary effect on the stomach is due to neutralization of gastric hydrochloric acid, promotion of mucosal defense mechanism and inhibition of the proteolytic enzyme pepsin.[4,5] Commonly used, over the counter antacids contain sodium bicarbonate, calcium carbonate, magnesium hydroxide, aluminum hydroxide and other excipients.[6] They are indicated in the treatment of duodenal and gastric ulcers, stress gastritis, gastro-oesophageal reflux disease (GERD), pancreatic insufficiency, non-ulcer dyspepsia, bile acid mediated diarrhoea, biliary reflux, constipation, osteoporosis, urinary alkalinisation and chronic renal failure as a dietary phosphate binder.[7]

In view of heavy commercial availability and affordability of antacids in Nigeria, its microbiological safety is of an important public health concern. Presence of objectionable organisms not only make them unsafe, but capable of changing the chemical, physical and organoleptic properties of the products and/or degrade the active ingredients.[2] It has been reported that some non-sterile preparations show microbial counts exceeding the USP acceptable limits and are found to contain objectionable organisms. These microbiological product qualities were influenced by change in pH, failure in the quality control process of raw material and product.[8-13] Udeze *et al*. reported the non-conformity of some brands of antacids assessed in Ilorin, Nigeria.[14] while Emejuru M.C *et al* revealed fungal and bacterial contaminants in more than 65 % of the total samples tested.[15] The organisms isolated were identified to be *Lactobacillus fermenti, Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium* spp, *Enterococcus* spp *and Streptococcus* spp, *Proteus, Klebsiella, Pseudomonas, Mucor* and *Aspergillus*.[15] In this study, we assessed the microbial quality of various

brands of antacid suspensions sold in Port Harcourt, possible presence of objectionable microorganisms and their susceptibility to commonly prescribed antibiotics.

MATERIALS AND METHOD

Culture Media

The media used in this study includes, Sabouraud Dextrose Agar, Plate count Agar, MacConkey Agar, Nutrient Agar, Cetrimide agar, Muller Hinton Agar (Titan Biotech Ltd India). The media were reconstituted according to manufacturer's specification.[16-18]

Antimicrobial Agents

The antimicrobial agents used were commercially prepared antibiotic disc from Abtek Biologicals Ltd; (Lot/Batch Number: RC09/P)

Antacid sample

Thirteen (13) brands of antacid suspension were randomly purchased from approved Pharmacies within Port Harcourt Metropolis, South-south Nigeria. Label information (batch numbers, manufacturing and expiry dates, National Agency for Food and Drug Administration and Control (NAFDAC) Registration Number were recorded and samples were transported to Pharmaceutical Microbiology laboratory, University of Port Harcourt for evaluation.

Pharmaceutical and organoleptic Assessment

All the samples were evaluated at the time of purchase. Prior to evaluation, they were properly inspected by checking their cap, seal, label and then disinfecting the caps and neck of the both bottles with 70% alcohol before and after opening. The colour, taste, and smell of each antacid sample were examined using the appropriate sense organ. The pH and sedimentation rate of the samples were performed in triplicate and the average data recorded for each sample.

pH determination

The pH of different antacid brands was determined using the Jenway UK pH meter, 3501 model. After effective agitation of antacid sample, an aliquot was dispensed into a test tube and the electrode part of the pH meter was dipped into it until the reading became stable. This was done in duplicate and average determination taken.

Sedimentation volume determination

Each antacid sample was adequately agitated, and 50 mL aseptically transferred from each bottle into 50 ml measuring cylinder and left undisturbed. Observations were made for twenty-eight days and the sedimentation volume calculated using the formula:

$$F = \frac{Vu}{Vo} \quad \text{..... Equation (1)}$$

Where V_u = Ultimate volume of sediment
V_o = Original volume of suspension.
F = Sedimentation volume

Microbiological Evaluation

Test sample preparation

Test samples were vigorously agitated and six (6), 10-fold serial dilution of each test suspension was prepared by diluting 1 ml volume into 9 ml of diluent.

Microbial enumeration and isolation

Total viable aerobic count for the enumeration and isolation of microorganisms were performed using spread plate technique on Plate count agar (PCA), Sabouraud dextrose agar (SDA) and MacConkey agar (MA) respectively as previously described.[19-21] The PCA and MA plates were incubated at 37 °C for 24 hours while SDA plates at 25 ^{o}C for 5 days. Uninoculated plates containing only the sterile media were used as blank to compare the different samples. After the incubation period, discrete colonies were counted using a colony counter and the total aerobic counts expressed as cfu/mL. The characteristic colonies were further purified and identified by their cultural, morphological and biochemical characteristics previously described.[16] Pure isolates were inoculated on agar slants and stored at 4 °C for future use.

Antibiotic Susceptibility Testing

Bacterial isolates were tested for their susceptibility to the commonly used antibiotics following the modified Kirby-Bauer disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) using standardized inoculums in Muller Hinton agar.[22] The commercially prepared antibiotic discs contained the following antibiotics: ceftriaxone 30 µg, cloxacillin 5 µg, ofloxacin 5 µg, erythromycin 5 µg, gentamicin 10 µg, cefuroxime 30 µg, ceftazidime 30 µg, amoxicillin/clavulanate 20/10 µg, nitrofurantoin 300 µg, ampicillin10 µg and ciprofloxacin 5 µg placed equidistance to each other. Uninoculated plates containing only the media and antibiotic disc were used as blank to compare the different samples. The plates were incubated in an inverted position at 37 ^{o}C for 24 hours and the diameters of the zones of growth inhibition around each of the antibiotic disks were measured to the nearest millimeter.

RESULTS AND DISCUSSION

Organoleptic properties

The organoleptic properties (colour, odour and taste) of samples analysed are presented in Fig. 1. All samples had a minty taste and odour (92.31 %) except product C5 with strawberry to taste and odour (7.69 %). Three colours identified varied from pink (53.85 %), white (38.46 %) to pale yellow (7.69 %). Use of colourants and flavourants as product excipients may have contributed to the colour and odour differences. However, these excipients are capable of supporting microbial growth and proliferation if not properly processed during manufacturing.

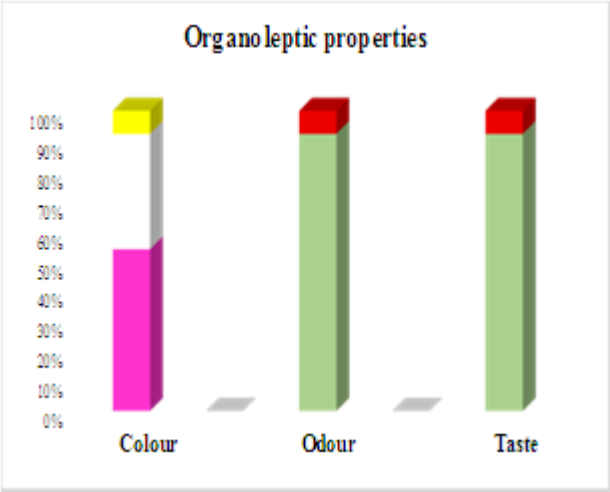

Fig. 1: Organoleptic properties of antacid samples.

Sedimentation volume

The result of sedimentation volume obtained from this study is shown in Table 1. The value ranged from 0.54 - 0.96 occurring in 35.7 % of samples, while 64.3 % did not show sedimentation after 28 days. According to Okoye and Lawal,[23] sedimentation volume is a quality assessment tool which provides information about the physical stability of pharmaceutical products. It is desirable that sedimentation volume of suspensions should fall between "0" and "1" in a scale of "0" to "1" and from the result; none of the samples had a sedimentation volume of less than 0.5. High volume of clear supernatants is sometimes inferred as a sign of poor product quality.[23]

Table 1: Sedimentation volume for each sample within 28days.

Sample Code	Sedimentation Volume (Days)				
	0-3	7	14	21	28
C1	0	0	0	0	0
C2	0	0	0	0	0
C3	0	0	0.98	0.96	0.94
C4	0	0	0	0	0
C5	0	0	0	0	0
C6	0.60	0.54	0.54	0.54	0.54
C7	0	0	0	0	0
C8	0	0	0.98	0.96	0.96
C9	0	0	0	0	0
C10	0	0	0	0	0
C11	0.74	0.64	0.64	0.62	0.62
C12	0	0	0	0	0
C13	0	0	0.82	0.82	0.82

pH

The result of the pH experiment is presented in **Fig. 2**. Product C6 was the most alkaline with a pH of approximately 9.45, while product C5 was the least with pH of about 7.43. As an important parameter in this study, it predicts the ability of the product to neutralize or reduce gastric hyperacidity and bringing succor to the user. As shown, the pH values of all the products were in the alkaline range. This result is in agreement with previous report stating that the product contains magnesium hydroxide.[23]

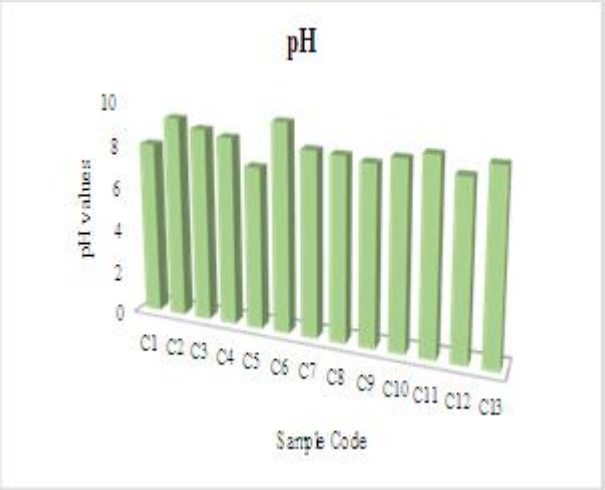

Fig. 2: pH of individual samples.

Microbiological Evaluation

All samples evaluated were contaminated with bacteria. Total viable cell counts of the tested antacid samples ranged between 1.1 x 10^2cfu/mL and 8.0 x 10^4cfu/mL (Table 2). A 92.3 % of the samples tested were within the acceptable bacterial count limit of 1.0 x 10^3 cfu/mL for non-sterile dosage forms except sample C1 with 8.0 x 10^4cfu/mL While 71 % of samples evaluated showed no evidence of objectionable bacterial growth, there was a 100 % *Escherichia coli* contamination present in the 30.77 % samples contaminated with objectionable bacteria (Fig. 3). The microbiological quality of antacid suspension samples studied seems to agree with the report by Sheikh *et al*. indicating that sterility is not necessarily a requirement in the official compendia for oral pharmaceutical dosage forms. The presence of microorganisms is worrisome because of the ability of these contaminant microorganisms to become pathogenic causing diseases such as vomiting, nausea, stomach cramps and gastrointestinal distress. *Escherichia coli*, an indicator of faecal contamination inhabit the respiratory, intestinal and urogenital tract of human and has been linked to toxin production within the human body that causes severe bloody diarrhea, abdominal cramps and hemolytic uremic syndrome in geriatrics and paediatrics.[24]. *Klebsiella pneumoniae* though contaminated with few of the samples tested (7.69 %) is a major public health concern (Fig. 3). It is opportunistic pathogen leading to nosocomial infections in the urinary tract, respiratory tract, lung, wound sites and septicaemia. Its occurrence has become more worrisome due to the emergence of extended-spectrum β-lactamases (ESBLs) and carbapenemase-producing strains.[25,26] Generally, presence of even a low level of pathogenic microorganisms, can lead to ineffective products, changes in their physical characteristics, cracking of emulsions, thinning of creams and fermentation of syrups and suspensions.[2] The great majority of the

microbiological contamination for non-sterile products could be attributed to the quality of raw materials, water to poor practices during product manufacturing and storage.[2] As a requirement therefore, operators would have to follow specific good manufacturing practices (GMP) such as raw material testing, equipment sterilization, appropriate gowning including training of personnel to ensure product safety. From this study, it could be suggested that contamination may have occurred during manufacturing, packaging, and/or during storage.

Table 2: Total viable cell counts of bacteria.

Sample code	Bacteria Cell Count (cfu/mL)
C1	8.0×10^4
C2	5.0×10^1
C3	5.2×10^2
C4	8.0×10^1
C5	1.2×10^2
C6	4.0×10^1
C7	8.0×10^1
C8	4.0×10^1
C9	1.1×10^2
C10	1.6×10^2
C11	5.0×10^1
C12	1.1×10^2
C13	1.1×10^2

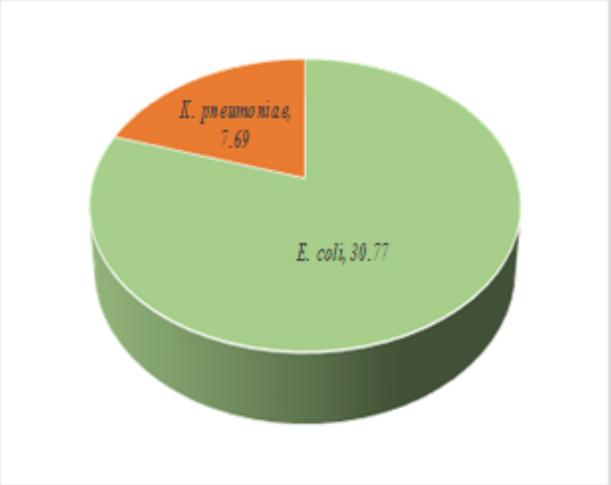

Fig. 3: Prevalence of objectionable bacteria in antacid brands.

Antibiotic susceptibility testing

The isolates *E. coli* and *K. pneumoniae* were tested for their susceptibility to commonly used antibiotics and the results of inhibition zones measured in millimeter are presented in Fig. 4 and 5.

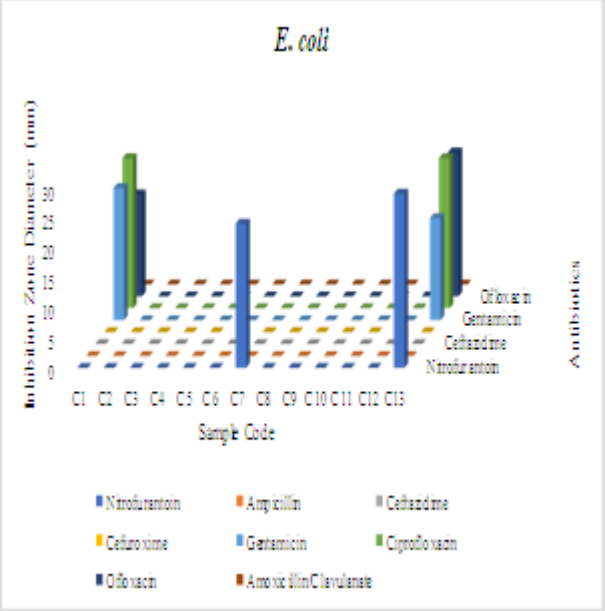

Fig 4: Antibiotic susceptibility test for *Escherichia coli.*

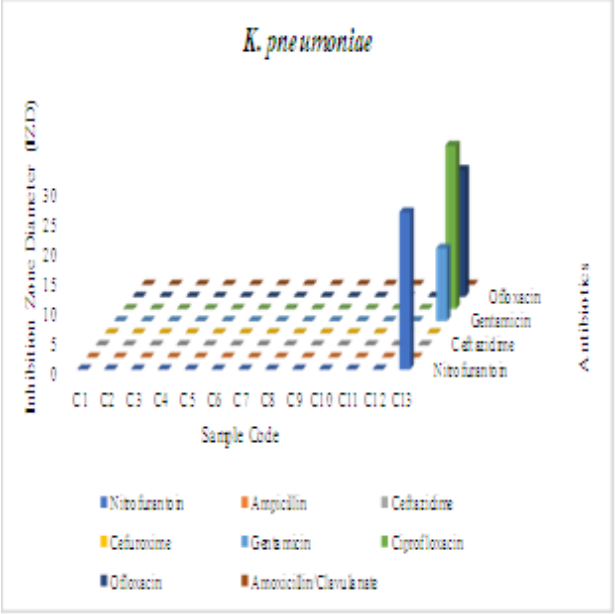

Fig. 5: Antibiotic susceptibility test for *Klebsiella pneumonia.*

From the result obtained, *Staphylococcus Spp* was resistant to the five antibiotics (erythromycin, augmentin, ceftazidine, ceftriaxone and cefuroxime) used except ofloxacin and gentamycin and this makes them extensively drug resistant.[27] Previous studies showed that ß-lactamase producing *S. aureus* had the highest percentage susceptibility to erythromycin, as against the result obtained in this study.[28] One of the major mechanisms of resistance to β-lactams is the expression of the enzymes, beta-lactamases such as penicillinase and cephalosporinase.[29]

There were obvious differences in terms of how *Escherichia coli* (although isolated from different antacid

samples) responded to the same antimicrobial agent. In general, *Escherichia coli* was resistant to majorly the cephalosporins and penicillins, but susceptible to fluoroquinolones, macrolides and nitrofurans. Looking at the individual samples, the results indicate that *E. coli* isolated from 20 % of the total examined were resistant to all the antibiotics and susceptible to two or fewer classes of antibiotics used. The same organism isolated from 60 % of the samples tested showed resistance to at least one antibiotic in three or more classes of the antibiotics used. Results from 20 % of the total sample showed resistance to all the classes of antibiotic used. This means that the *E. coli* is extensively drug resistant, multidrug resistant and pan-drug resistant to the antibiotic tested.[27,30,31] and in agreement with the previous studies on antimicrobial-resistance of *E. coli* isolated from children in Shahid Sadoughi hospital of Yazd by Ayatollahi *et al*.[32] showing varying response to the antibiotics tested. With respect to *Klebsiella pneumoniae*, resistant was recorded against ceftazidime, cefuroxime, ampicillin and Augmentin, but was susceptible to nitrofurantoin, gentamicin, ciprofloxacin and ofloxacin. This means that the isolate is multidrug resistant. The resistant pattern recorded in this study may be attributed to several factors including the misuse and overuse of these antimicrobials in our setting, the acquisition of resistance through mutations in some of their genes when they are exposed to an antibiotic.[33] Previous report indicates that this resistance, natural or acquired, can spread to other bacterial species since bacteria can easily exchange genetic material from one to another, even if they are from different species.[33,34]

The diameter of the zone of inhibition is related to the susceptibility of the isolate and to the rate of diffusion of the drug through the agar medium as previously reported.[35,36] Susceptibility to at least one agent in three or more antimicrobial categories was considered as being multidrug resistant (MDR). Non-susceptibility to at least one agent in all but two or fewer antimicrobial categories was considered as being extensively drug resistance (XDR). Non-susceptibility to all agents in all the antimicrobial categories was considered as being pan-drug resistance (PDR).[37,38]

CONCLUSION

Microbiological contamination of non-sterile pharmaceutical liquid products has long been a major concern when discussing public health risks and drug efficacy. The study provided information on the microbial quality and resistant patterns of some antacid suspensions marketed in Port Harcourt Metropolis in order to help in management of real-life cases of health problems precipitated from consumption of contaminated antacid suspensions. The different brands of antacid suspensions studied, revealed the presence of objectionable organisms with *Escherichia coli* being the most prevalent followed by *Klebsiella pneumoniae*. The organisms showed different patterns of resistance ranging from Extensive drug resistance to Pan-drug resistance. One of the most important areas in pharmaceutical process control is the development of systems to control the number, survival, and proliferation of microorganisms during manufacturing of non-sterile and sterile pharmaceutical products. Therefore, there is the need to maintain good standards by adhering to Good Manufacturing Practice as a way of eliminating undesirable microorganism from products.

REFERENCES

1. Jimenez L: Microbial Contamination Control in the Pharmaceutical Industry: Taylor & Francis, 2004.
2. Ratajczak M, Kubicka MM, Kamińska D, Sawicka P, Długaszewska J: Microbiological quality of non-sterile pharmaceutical products. *Saudi Pharmaceutical Journal : SPJ*, 2015; 23(3): 303-307.
3. Denyer SP, Hodges NA, Gorman SP, Hugo WB: Hugo and Russell's pharmaceutical microbiology, 7th edn. Oxford: Blackwell, 2003.
4. Mejia A, Kraft WK: Acid peptic diseases: pharmacological approach to treatment. *Expert review of clinical pharmacology*, 2009; 2(3): 295-314.
5. Tarnawski A, Ahluwalia A, Jones MK: Gastric cytoprotection beyond prostaglandins: cellular and molecular mechanisms of gastroprotective and ulcer healing actions of antacids. *Current pharmaceutical design*, 2013; 19(1): 126-132.
6. Ravisankar P, Koushik O, Reddy A, Eswar kumar U, Anvith P, Pragna P: A Detailed Analysis on Acidity and Ulcers in Esophagus, Gastric and Duodenal Ulcers and Management. *IOSR-J of Dental and Med Sci.*, 2016; 15(1): 94-114.
7. Maton PN, Burton ME: Antacids revisited: a review of their clinical pharmacology and recommended therapeutic use. *Drugs*, 1999; 57(6): 855-870.
8. Hossain M, Shamim A, Rahman MZ: Quantitative Examination of Aerobic Bacteria and Fungi in Locally Available Antacid Suspension and Possible Contamination by Specified Bacteria. *Pakistan J of Biol, Sci.*, 2004; 7(11): 2014-2017.
9. Kilani AM, KW O: Microbiological Quality of Selected Non-Sterile Pharmaceutical Products Sold in Retail Outlets in Dutsinma Metropolis, Katsina State, Nigeria. *Public Health Dev Ctries*, 2017; 3(1): 339-346.
10. Moniruzzaman M, Ashrafi FF, Mia Z: Qualitative and Quantitative Microbiological Studies of Antacid And Paracetamol Suspensions from Different Drugstores of Dhaka. *Dhaka Univ J Biol Sci.*, 2012; 21(1): 105-107.
11. Jahan Urmi N, Noor R: Microbiological profile and the anti-bacterial traits of commonly available antacid suspensions in dhaka metropolis, 2014; 6.
12. Kabir MS, MD H: Microbiological Quality Assessment of Vitamin B Syrups and Antibiotic Susceptibility Profile of the Isolated Escherichia coli. *IOSR-J Pharm & Biol Sci.*, 2013; 8(4): 61-64.

13. Ibezim C, Nwosu C, Ogbu H: Bacteriological Screening of Paediatric Cough Syrups Marketed Within Port Harcourt Metropolis, South-South Nigeria. *Int J Pharm Sci Drug Res.*, 2018; 10(2): 65-70.
14. Udeze AO, Jayeoba TM, Innocent-Adiele HC, Okerentugba PO, Nwanze JC, Onoh CC, IO O: Bacteriological Assessment of Some Selected Antacid Suspension Products in Nigeria. *N Y Sci J.*, 2012; 5(5): 28-32.
15. Emejuru MC, Ojiegbe GC, Azi S, NB N: Microbiological Load of Selected Oral Liquid Pharmaceuticals. *Int J, Comm Res.*, 2013; 2(3): 39-45.
16. Sandle T: 5 - Microbiological culture media. In: *Pharmaceutical Microbiology*. Oxford: Woodhead Publishing; 2016; 47-61.
17. Cundell AM: Review of the media selection and incubation conditions for the compendial sterility and microbial limit tests, 2002; 28.
18. Ibezim C, Nwosu C, Ogbu H: Bacteriological Screening of Paediatric Cough Syrups Marketed Within Port Harcourt Metropolis, South-South Nigeria. *International Journal of Pharmaceutical Sciences and Drug Research*, 2018; 10(2): 65-70.
19. Razvi N, Awan R, Naqvi S, Anjum DF, Hussain Z, Farooqi S: Estimation of Microbial Contamination in Various Active Pharmaceutical Ingredients and Excipients, 2014; 3.
20. Adeola A, MI O, IA A: Microbial quality of some non-sterile pharmaceutical products sourced from some retail pharmacies in Lagos. *Afr J Microbial Res.*, 2012; 6: 4903-4907.
21. Cheesbrough M: District Laboratory Practice in Tropical Countries, 2 edn. Cambridge: Cambridge University Press, 2006.
22. Institute CLS: Performance Standards for Antimicrobial Susceptibility Testing, Nineteenth informational supplement M100-S19, Wayne, Pa, USA. *Clinical and Laboratories Standards Institute*, 2009.
23. Okoye IE, Lawal OR: Comparative assessment of price and quality of liquid antacids in Nigeria. *Pharm Methods*, 2017; 8: 54-61.
24. Paton JC, Paton AW: Pathogenesis and Diagnosis of Shiga Toxin-Producing Escherichia coli Infections. *Clinical Microbiology Reviews*, 1998; 11(3): 450-479.
25. Biendo M, Canarelli B, Thomas D, Rousseau F, Hamdad F, Adjide C, Laurans G, Eb F: Successive Emergence of Extended-Spectrum β-Lactamase-Producing and Carbapenemase-Producing <em>Enterobacter aerogenes</ em> Isolates in a University Hospital. *Journal of clinical microbiology*, 2008; 46(3): 1037.
26. Chung PY: The emerging problems of Klebsiella pneumoniae infections: carbapenem resistance and biofilm formation. *FEMS Microbiology Letters*, 2016; 363(20): fnw219-fnw219.
27. Falagas ME, Karageorgopoulos DE: Pandrug Resistance (PDR), Extensive Drug Resistance (XDR), and Multidrug Resistance (MDR) among Gram-Negative Bacilli: Need for International Harmonization in Terminology. *Clinical Infectious Diseases*, 2008; 46(7): 1121-1122.
28. Akindele A, Adewuyi I, Adefioye O, Adedokun S, Olaolu A: Antibiogram and Beta-Lactamase Production of Staphylococcus aureus Isolates from Different Human Clinical Specimens in a Tertiary Health Institution in Ile-ife, Nigeria. *American-Eurasian J of Sci Res.*, 2010; 5(4): 230-233.
29. Zeng X, Lin J: Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria. *Frontiers in Microbiology*, 2013; 4: 128.
30. Ali A, Hasan R, Jabeen K, Jabeen N, Qadeer E, Hasan Z: Characterization of mutations conferring extensive drug resistance to Mycobacterium tuberculosis isolates in Pakistan. *Antimicrob Agents Chemother*, 2011; 55(12): 5654-5659.
31. Mathur P, Singh S: Multidrug resistance in bacteria: a serious patient safety challenge for India. *J Lab Physicians*, 2013; 5(1): 5-10.
32. Ayatollahi J, H Shahcheraghi S, Akhondi R, Soluti ss: Antibiotic Resistance Patterns of Escherichia coli Isolated from Children in Shahid Sadoughi Hospital of Yazd, 2013; 3.
33. Woodford N, Ellington MJ: The emergence of antibiotic resistance by mutation. *Clinical Microbiology and Infection*, 2007; 13(1): 5-18.
34. Munita JM, Arias CA: Mechanisms of Antibiotic Resistance. *Microbiology spectrum*, 2016, 4(2): 10.1128/microbiolspec.VMBF-0016-2015.
35. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ: Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clinical Infectious Diseases*, 2009; 49(11): 1749-1755.
36. Balouiri M, Sadiki M, Ibnsouda SK: Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 2016; 6(2): 71-79.
37. Magiorakos AP, 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. *Clinical Microbiology and Infection*, 2012; 18(3): 268-281.
38. Basak S, Singh P, Rajurkar M: Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study. *Journal of Pathogens*, 2016; 2016: 4065603.