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Research Article
Organoleptic Property and Bacteriological Analysis of Cough Syrups Sold in Sokoto Metropolis, North-Western Nigeria

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Abstract

Background and Objective: Syrups are dosage forms of choice in paediatric and geriatric patients having respiratory diseases with symptoms such as cough. They are non-sterile preparations to which there is a specified limit for microbial load and organoleptic property they must conform to. This research investigated the organoleptic property and bacteriological analysis of cough syrups sold in the Sokoto metropolis. **Materials and Methods:** Ten brands of cough syrup samples were subjected to an organoleptic property test using some human sense organs such as eyes for colour, tongue for taste and nose for the smell. Constitutive bacteria were isolated and characterized using standard methods. Antibiotics susceptibility profiles of the isolated bacteria to four antibiotics namely tigecycline, cephalexin, ampicillin/sulbactam and meropenem were determined by subjecting them to antibiotics susceptibility tests using the agar disc diffusion method. **Results:** All the samples showed bacterial contamination with values ranging from 1.0×10^2 to 1.07×10^4 CFU mL^{-1}, with 60% of the samples containing bacterial contamination above the USP specified limit (10^2 CFU mL^{-1}), while only 20% of the 40% within the specified limit passed the bacteriological evaluation with the presence of some objectionable bacterial organisms which made the syrups unfit for consumption. The potentially pathogenic organisms isolated were *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Escherichia coli*, *Salmonella* spp., *Bacillus* spp. and *Klebsiella* spp., with *Bacillus* spp., having the highest occurrences (7 out of 10 samples) among the bacterial contaminants. **Conclusion:** All the isolates were sensitive to tigecycline but showed varied resistance to cephalexin, ampicillin/sulbactam and meropenem. Poor adherence to good manufacturing practices and poor handling and storage of pharmaceutical products from the part of the retailer might have led to microbial contamination. This calls for the need for strict adherence to good manufacturing practices and proper storage and handling of the products.

Key words: Organoleptic property, bacteriological analysis, antibiotic susceptibility, cough syrup, Sokoto metropolis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Syrups are a concentrated solution of sugar mixed in water or other aqueous liquid. They are used as a vehicle for medicine with flavour incorporated. They are dosage forms of choice in paediatric and geriatric patients in respiratory diseases symptom such as cough[1,2]. Syrups are intended to serve as a pleasant-tasting vehicle for medicinal substances, in a bid to improve patient compliance and acceptability[3].

A high concentration of sugar present in syrup (60-80%) serves both protagonist and antagonistic role in the stability of the preparation. The sugar reduces the water activity (A_w) of the preparation, impact viscosity thus making it unsuitable for microbial proliferation, while the sugar may also serve as a food substrate for the microbes in addition to other additives present in the preparation[4].

Syrups are non-sterile preparation in which there is a specified limit for the microbial load which they must conform to as per pharmacopoeia specifications, the total viable count of permissible bacteria (i.e., non-pathogenic bacteria) must not exceed 10^2 CFU mL^{-1} while yeast and moulds should not exceed 10^1 CFU mL^{-1}. Perhaps liquid pharmaceutical preparations are to be free of *E. coli* and other coliforms, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and other pathogens following the Reports of Mixed Working Committee of Official Laboratory and Drug Control Services and a section of the International Pharmaceutical Federation[5,6].

Microbial contamination of cough syrup can be traced from the raw materials used, the equipment used in the manufacturing process, the environment, the personnel and process such as packaging and during storage of the finished product. Several factors aid the microbial load of syrups which include non-compliance with Good Manufacturing Practice, low-quality raw materials and most importantly low sucrose concentration[7,8]. Microbial load should therefore be monitored from raw materials to the finished products to determine the source of contamination. Bacterial contamination affects the physicochemical properties[2] and therapeutic properties of the preparation and this may lead to loss of confidence on the part of the patient[5]. Patients being treated with contaminated cough preparation are at risk of developing secondary complications, the severity being a factor of the age of the patient and the immune status[9].

Economic loss due to unstable and unaesthetically products is a major concern[5]. Health hazards that depend on the amount ingested and other patient-related factor includes, nosocomial infection, salmonellosis, urinary tract infection, gastroenteritis, abdominal discomfort and diarrhoea due to toxin released from the bacteria[10,11]. This is of particular concern in paediatric and geriatric patients with less efficient immunity who may acquire infection which are difficult to treat and which takes a longer time to respond to treatment. Severe infections with *Klebsiella* and *Bacillus* spp., have been reported in immune-compromised patient[8]. *Staphylococcus aureus* may cause a significant deterioration in the health of immune-compromised adult patients and infant with less developed immunity[12]. However, organisms such as *Salmonella* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* have been recommended as indicators of pathogenic microorganism contaminants of syrups[13,14].

Varied resistance to commonly prescribed antibiotics against bacteria isolated from contaminated syrup and oral preparation is posing a great hazard and a loss in the fight against infectious disease.

Multi-drug resistance of *Klebsiella pneumonia* and extensive drug resistance of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* isolated from cough syrup, have been reported[2,15].

The high temperature experienced for most time of the year in Sokoto may lead to an increase in the water activity of syrups due to dissolution of the sugar present in the preparation could encourage microbial spoilage and product instability, a factor associated with lack of proper storage condition in retail outlets.

Poor manufacturing practices, poor storage conditions and increased reports of disease emanating from consumption of contaminated syrup necessitate a periodic evaluation of cough syrup as it is the preferred dosage form in both infant and adult patients. Thus this study was carried out on the organoleptic properties, bacteria load (bioburden) of and antibiotic susceptibility profiles of the isolated bacteria from cough syrups sold in Sokoto metropolis, North-Western Nigeria.

MATERIALS AND METHODS

Study area: This study was carried out in the Microbiology Laboratory of the Department of Pharmaceutics and Pharmaceutical Microbiology, Usmanu Danfodiyo University, Sokoto-Nigeria, between March and September, 2021.

Culture media and antibiotics: The media used include, Nutrient broth, Nutrient agar, Cetrimide agar, MacConkey agar, Mannitol salt agar, Muller-Hinton agar, Triple sugar iron agar and Simon citrate agar (Biotech, UK). The media were reconstituted according to the manufacturer's instruction. The

following antibiotics were used, cephalexin (30 µg), ampicillin/sulbactam (20 µg), tigecycline (15 µg), meropenem (5 µg) (Oxoid, UK).

Sample collection: Ten brands of commonly sold cough syrup in Sokoto were purchased randomly in duplicates from registered premises within Sokoto metropolis, North-Western Nigeria. Transported and evaluated within 24 hrs of procurement in the Pharmaceutical Microbiology Laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The samples were checked for vital information such as NAFDAC registration numbers, names of the manufacturer and expiry dates.

Organoleptic properties: Colour, odour and taste of the cough syrup samples were undertaken using appropriate sense organs.

Bacteriological analysis

Bioburden: One mL of the sample was withdrawn and diluted in 9 mL of sterile normal saline then mixed thoroughly, then 0.1 mL of the serially diluted sample was then plated on the nutrient agar plate, MacConkey agar plate, mannitol salt agar plate and Cetrimide agar plate in duplicate then incubated at 35°C for 24-48 hrs. The resulting colonies were characterized and isolated organisms identified using standard methods[2,9,14].

Antibiotic susceptibility testing using agar disc diffusion method (Kirby-Bauer method): Muller-Hinton-agar was prepared according to the manufacturer's directive, sterilized and poured in sterile Petri dishes and allowed to solidify at room temperature. Then inoculated with the already standardized organism (using 0.5 McFarland standard) by streaking method, the antibiotic disc impregnated with the desired concentration of antibiotic was placed at equidistance to each other using sterile forceps and incubated at 35±2°C for about 24 hrs. Zone of inhibitions was measured using a ruler and values obtained were used to categorize the isolated bacteria into sensitive and resistant groups using official guidelines[15].

RESULTS

Organoleptic properties: The result of organoleptic properties of the tested samples showed different colour variations ranging from brick-red, brown to pink, while the odour varies from minty, fruity to caramel and the taste showed variation from minty to fruity as presented in Table 1.

Table 1: Organoleptic properties of cough syrups sold in Sokoto metropolis

Samples	Colours	Odour	Taste
S_1	Brick red	Minty	Fruity/minty
S_2	Brown	No distinct odour	Sour
S_3	Pink	Fruity	Minty-sweet
S_4	Brown	Caramel	Mildly-sweet
S_5	Brick red	Pleasant	Less-fruity
S_6	Pink	Minty	Less-minty
S_7	Deep-pink	Aromatic	Less-fruity
S_8	Dark-brown	Minty	Minty-sweet
S_9	Brown	Caramel	Less-sweet
S_{10}	Pink	Minty	Minty

Table 2: Total viable counts of bacteria from cough syrups sold in Sokoto metropolis

	Bacterial counts (CFU mL^{-1})			
Samples	NA	MC	CT	MN
S_1	2.0×10^2	1.0×10^2	-	-
S_2	2.0×10^2	-	-	-
S_3	1.0×10^2	-	1.0×10^2	-
S_4	1.3×10^4	-	-	-
S_5	1.0×10^2	-	-	-
S_6	1.8×10^3	-	-	-
S_7	2.0×10^3	-	-	-
S_8	6.7×10^3	-	6.0×10^2	-
S_9	6.0×10^2	-	-	-
S_{10}	1.07×10^4	-	-	-

NA: Nutrient agar, MC: MacConkey agar, CT: Cetrimide agar, MN: Mannitol salt agar and -: Zero

Bacteriological analysis

Bioburden: The result of the bacteria load of the cough syrup samples showed total aerobic count values ranging from 1.0×10^2 to 1.07×10^4 CFU mL^{-1} on nutrient agar. Only S_1 had growth with the count of 1.0×10^2 CFU mL^{-1} on macConkey agar with S_3 and S_8 having values of 1.0×10^2 and 6.0×10^2 CFU mL^{-1}, respectively on cetrimide agar. No growth was found on mannitol salt agar in Table 2. The result further showed the distribution of seven bacterial contaminants in the cough syrup samples namely *Bacillus* spp., *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella* spp. and *Salmonella* spp., S_8 had the highest number (5) of bacterial contaminants followed by S_6 and S_{10} with 3 bacterial contaminants while S_5 had the least value of 1 bacterium as a contaminant. The majority (7 out of 10) of the samples had *Bacillus* spp., as a contaminant followed by *Streptococcus* spp. (6 out of 10) while *E. coli* (1 out of 10) was the least contaminant found only in S_8 with the highest number of contaminants in Table 3.

Antibiotic susceptibility testing: Table 4 showed the results of antibiotic susceptibility testing of isolated bacteria from the cough syrup samples. All isolated bacteria were Sensitive (S) to Tigecycline (TGC), only three (*Pseudomonas* spp.,

Table 3: Distribution of bacterial contaminants in cough syrups sold in Sokoto metropolis

Samples	BS	EC	PS	SS	ST	KS	SA	No. of contaminant
S_1	-	-	+	+	-	-	-	2
S_2	+	-	-	-	+	-	-	2
S_3	-	-	-	+	-	+	-	2
S_4	+	-	-	-	+	-	-	2
S_5	+	-	-	-	-	-	-	1
S_6	+	-	-	+	+	-	-	3
S_7	-	-	-	+	+	-	-	2
S_8	+	+	+	-	+	-	+	5
S_9	+	-	-	-	-	+	-	2
S_{10}	+	-	-	-	+	-	+	3

BS: *Bacillus* spp., EC: *Escherichia coli*, PS: *Pseudomonas* spp., SS: *Staphylococcus* spp., ST: *Streptococcus* spp., KS: *Klebsiella* spp., SA: *Salmonella* spp., +: Present and -: Absent

Table 4: Antibiotic susceptibility test on bacteria from cough syrups sold in Sokoto metropolis

	Zone of Inhibition (mm)			
Bacterial isolates	TGC (15 µg)	CL (30 µg)	SAM (20 µg)	MEM (10 µg)
Pseudomonas spp.	21 (S)	16 (S)	-	-
Staphylococcus spp.	19 (S)	25 (S)	-	-
Streptococcus spp.	24 (S)	20 (R)	-	-
E. coli	20 (S)	16 (R)	-	-
Klebsiella spp.	25 (S)	14 (R)	-	-
Bacillus spp.	23 (S)	12 (S)	-	-
Salmonella spp.	26 (S)	13 (R)	-	-

TGC: Tigecycline, CL: Cephalexin, SAM: Ampicillin/Sulbactam, MEM: Meropenem, -: No growth, S: Sensitive and R: Resistant

Staphylococcus spp. and *Bacillus* spp.) were sensitive (S) to cephalexin (CL) while four isolated bacteria namely *Streptococcus* spp., *E. coli*, *Klebsiella* spp. and *Salmonella* spp., were Resistant (R) to the same cephalexin (CL). However, no growth was found on ampicillin/Sulbactam (SAM) and Meropenem (MEM) for all isolated bacteria.

DISCUSSION

The result of organoleptic property shows varied changes in colour, taste and odour indicating spoilage which might be a result of poor manufacturing practices and/or poor storage. The present study also showed that the cough syrup samples contained varying levels of bacterial contaminants. Samples S_1, S_2, S_3 and S_5 had bacteria load below the limit specified in the pharmacopoeia that the total viable count of permissible bacteria (i.e., non-pathogenic bacteria) must not exceed 10^2 CFU mL^{-1} while yeast and moulds should not exceed 10^1 CFU mL^{-1} [6], samples S_1 and S_3 despite having bio-burden within the permissible limit, were contaminated with objectionable organisms namely *Staphylococcus* spp. and *Pseudomonas* spp., in S_1 and *Klebsiella* spp. and *Staphylococcus* spp., in S_3 thus making them unfit for consumption. Objectionable bacteria specified by the USP are *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. Their presence even below the specified limit makes the products unfit for consumption. Since samples S_2 and S_5 satisfied bacteriological quality control tests for non-sterile preparation and were also not found wanting in organoleptic property tests, they are therefore likely fit for consumption.

The levels of contamination in samples S_4, S_6, S_7, S_8, S_9 and S_{10} in this study were higher than those reported in the study carried out by Anie and Okafo[7] with values above the specified limit. This might be due to poor adherence to Good Manufacturing Practices (GMP). Sample S_8 was heavily contaminated with Pseudomonas spp with a total aerobic count of 6.0×10^2, which is the highest count on Cetrimide agar. This result is similar to that of Ibezim *et al.*[2], where *Pseudomonas aeruginosa* was equally reported as a bacterial contaminant of syrups. *Pseudomonas aeruginosa* are opportunistic organisms that after improper washing of production machines or equipment may remain behind and contaminate subsequent production batches. A larger percentage of *Bacillus* spp., obtained in the current study is supported by the claim that they are the most predominant bacteria in non-sterile preparation due to their ubiquitous nature[4,5].

Isolation of other bacteria namely *Staphylococcus* spp., *Pseudomonas* spp., *Escherichia coli*, *Klebsiella* spp. and *Salmonella* spp., in the current research is of great concern. This raise eyebrow because they are potentially fatal considering the extensive use of cough syrup preparations in both paediatric and geriatric patients[2]. *Staphylococcus* spp.,

has been implicated in skin infection while *Pseudomonas* spp., are culprits in some inflammation disorders such as sepsis, while *Escherichia coli* have been implicated in paediatric diarrhoea. *Klebsiella pneumonia* is a major cause of infective pneumonia and *Salmonella* is causative of salmonellosis and *Bacillus subtilis* have been reported in some cases of food poisoning all of which are fatal in those with weaker immune system[2,13].

The routes of contamination have shown that the bacterial contaminants may likely be from personnel, water, manufacturing environment, equipment and packaging processes. The contamination can therefore be greatly reduced by stringent control of the manufacturing process and good quality control of raw materials and water used in production. Even though syrups generally have high sucrose content of about 65% that is inhibitory to microbial growth, poor storage conditions due to high temperature can lead to the dissolution of the sugar thus increasing the water activity of the syrup and making it susceptible to bacterial contamination. Proper storage conditions therefore should be encouraged to prevent spoilage of products even if they complied with the manufacturer's specification, to prevent economic loss due to spoilage.

The resistance to a beta-lactam antibiotic and beta-lactamase inhibitor poses a challenge since it is commonly used in health settings as a first-line antibiotic. Having four isolated bacteria (*Streptococcus* spp., *E. coli*, *Klebsiella* spp. and *Salmonella* spp.) resistant to cephalexin in this study, is of concern as it poses great challenges to achieving the positive clinical outcome and economic loss.

A previous study has shown *Klebsiella pneumonia* to behave multidrug resistance while *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were reported as having extensive drug resistance in a study carried out on bacteria isolated from cough syrups[2]. This corroborates the result of the current study where *Klebsiella* spp. and *E. coli* were resistant to cephalexin. Efficacy of both meropenem and ampicillin/sulbactam to out-rightly suppress the growth of all the bacterial contaminants might be due to non-prescription of meropenem and synergistic effect of ampicillin/sulbactam, while the sensitivity of the isolates to tigecycline may point to the fact that it is not widely available or prescribed in the region.

CONCLUSION

It could be deduced from the result that sample S_5 appeared to be the best and safest product among the ten products tested with acceptable organoleptic properties, total viable bacteria count not exceeding 10^2 CFU mL^{-1} with one bacterial contaminant (*Bacillus* spp.) which is not objectionable. Other samples failed in one way or the other in organoleptic property and bacteriological tests with samples S_1 and S_3 having objectionable organisms such as *Staphylococcus* spp., *Pseudomonas* spp. and *Klebsiella* spp., capable of causing health hazards. *Streptococcus* spp., *E. coli*, *Klebsiella* spp. and *Salmonella* spp., isolated from the cough syrup samples were resistant to cephalexin and this is of concern as it poses great challenges to achieving the positive clinical outcome and economic loss. It is therefore recommended that good manufacturing practices are put in place from quality control of raw materials to packaging of the product and proper usage of effective and efficient preservatives to reduce bio-burden, proper storage to forestall physical and microbial spoilage and good hygiene on the part of personnel is important to prevent contamination during handling of the product.

SIGNIFICANCE STATEMENT

This study discovered the level of contamination of cough syrups sold in Sokoto-Nigeria that can be beneficial for quality control of pharmaceutical products. The study will help researchers to uncover the critical areas of drug contamination that many researchers were not able to explore. Thus a new theory on drug management may be arrived at.

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REFERENCES

1. Irwin, R.S. and J.M. Madison, 2000. The diagnosis and treatment of cough. N. Engl. J. Med., 343: 1715-1721.
2. Ibezim, C.N.E., C.N. Nwosu and H.I. Ogbu, 2018. Bacteriological screening of paediatric cough syrups marketed within Port Harcourt metropolis, South-South Nigeria. Int. J. Pharm. Sci. Drug Res., 10: 65-70.
3. Allen, L.V., N.G. Popovich and H.C. Ansel, 2011. Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. 9th Edn., Lippincott Williams and Wilkins, Baltimore, United States, ISBN-13: 978-0-7817-7934-0, Pages: 710.

4. Sudeshika, S.H.T., G.J. Panagoda and I.U.K. Tennakoon, 2014. Microbiological quality of paediatric oral liquid drug formulations during consumption. Int. J. Sci. Res. Publ., Vol. 4.
5. Ratajczak, M., M.M. Kubicka, D. Kamińska, P. Sawicka and J. Długaszewska, 2015. Microbiological quality of non-sterile pharmaceutical products. Saudi Pharm. J., 23: 303-307.
6. Mukhtar, G.L., M.D. Mukhtar and A.M. Magashi, 2019. Assessment of microbial contamination in Pediatric oral liquid formulations marketed in Katsina State, Nigeria. J. Pure Appl. Sci., 12: 657-662.
7. Anie, C.O. and S.E. Okafo, 2020. Microbiological evaluation of some oral antacid suspensions sold in delta state, Nigeria. J. Appl. Sci. Environ. Manage., 25: 283-285.
8. Eziuzo, O.S. and C. Amarauche, 2017. Preliminary studies on the suspending properties of *Sida acuta* gum in paracetamol suspension. World J. Pharm. Pharm. Sci., 6: 302-313.
9. Anifowoshe, R.A., I.O. Morrison and A.A. Isaac, 2012. Microbial quality of some non-sterile pharmaceutical products sourced from some retail pharmacies in lagos, Nigeria. Afr. J. Microbiol. Res., 6: 4903-4907.
10. Opoku, S. and I. Nyanor, 2019. Qualitative and quantitative microbiological studies of paediatric artemether-lumefantrine dry powders and paracetamol syrups obtained from selected drug stores in Accra, Ghana. J. Trop. Med., Vol. 2019. 10.1155/2019/7062016.
11. Osungunna, M., M. Mba and O. Adebajo, 2016. Evaluation of microbial quality of selected blister-packed paracetamol tablets and paracetamol syrups marketed in Nigeria. Afr. J. Clin. Exp. Microbiol., 17: 151-158.
12. Biraima, R.E., S.E. Mohamed, M.A. Hajo, A.M. Elnazeer and M.A. Hussain, 2016. Microbiological quality assessment of antacid suspensions available in Sudan. Pharma Innov. J., 5: 17-19.
13. Gad, G.F.M., R.A.I. Aly and M.S.E. Ashour, 2011. Microbial evaluation of some non-sterile pharmaceutical preparations commonly used in the Egyptian market. Trop. J. Pharm. Res., 10: 437-445.
14. Obuekwe, I.F. and F. Eichie, 2006. The presence of microorganisms in some common excipients used in tablet formulation. Acta Poloniae Pharma. Drug Res., 63: 121-125.
15. Nassar, M.S.M., W.A. Hazzah and W.M.K. Bakr, 2019. Evaluation of antibiotic susceptibility test results: How guilty a laboratory could be? J. Egypt. Public Health Assoc., Vol. 94. 10.1186/s42506-018-0006-1.