Contents list available at Journal of e-Science Letters

Journal of e-Science Letters

www.scienceletters.researchfloor.org

Biohazards Signatures of Cough Syrup Isolates from Patent Medicine Shops, Akungba-Akoko Metropolis

Oludare Temitope Osuntokun & Adekahunsi, Agnes Oluwasimbo

Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

ABSTRACT

The aim of this study is, to isolate, identify and characterize microbial analysis & biohazards signatures of cough syrup isolates from patent medicine shop in Akungba-Akoko metropolis and determined the antibiotics pattern of the isolates from cough syrup. Microbial bioharzard of cough syrup can occur at various stages of the manufacturing process, including raw material handling, storage, and packaging. Microorganisms such as bacteria, fungi, and viruses can survive and multiply in the cough syrup, leading its contamination. For this reason, this study was conducted to investigate the quality and safety of cough syrup from patent medicine shops using a combination of conventional method and molecular techniques. Different brands of eigth (8) cough syrups were collected from different patent medicine shop in Akungba-Akoko and the pour plate technique was performed to enumerate the microbial contaminant from the collected samples. Bacterial isolates were identified using Bergeys Manual of Determinative Bacteriology based on the results obtained from microscopic examination, cultural and morphological examination and biochemical tests. The antibiotics susceptibility patterns of the isolates were determined. Using an Ultraviolet spectrophotometer the growth dynamics and killing time of the cough syrup isolates were also determined. The 16s molecular sequencing was used to validate the bacteria isolates after the conventional method of analysis. The result showed that 75% of organisms isolated were Gram-negative, while the remaining 25% were Gram-positive which includes Staphylococcus aureus (2), Bacillus spp (2), Proteus spp (4), Citrobacter freundii (1), Enterobacter spp (3)., Klebsiella spp.(1) and Salmonella spp.(3), Streptococcus spp.(2). The results of this study showed that the majority of the cough syrup isolates were resistant to different antibiotics; 62% of the Gram-negative microorganisms identified were resistance to the antibiotics used, while 20% of the Gram-positive microorganisms were resistant to the antibiotics used. The findings of this study highlight the need for improved regulation and oversight of patent medicine shops in Nigeria, to ensure the quality and safety of healthcare products and services for consumers. By using a combination of advanced techniques, this study provides valuable insights into the quality and safety of cough syrup products sold in patent medicine shops in Akungba-Akoko metropolis. These findings can inform policy and practice in the healthcare sector, contributing to the improvement of public health and safety in Nigeria.

Keywords: *Microbial analysis , hazards contaminant*

1.0 INTRODUCTION

Coughing is the rapid expulsion of air typically from the lungs in order to clear it of fluid, irritants, foreign particles and microbes (1; 2). In children, it could be productive or unproductive and often the most common reasons for which parents seek medical attention for their children (3). According to (4) Cough is also recognized as a defense reflex mechanism, with three phases: an inspiratory phase; a forced expiratory effort against a closed glottis; opening of the glottis, with subsequent rapid expiration, that generates a characteristic cough sound. Physiologists make the important distinction between cough and the closely related defense expiratory reflex, which does not result in a cough.

Received 10 January 2024
Revised 12 February 2024
Accepted 04 March 2024
Available Online 18 April 2024

Corresponding Authors: ***Oludare Temitope Osuntokun***
Email: ***oludare.osuntokun@aaua.edu.ng***

https://doi.org/10.51470/eSL.2024.5.1.07

Almost everybody has had cough after a common cold, which typically lasts 1–3 weeks (5; 6). The protective nature of cough is well illustrated by the complications of cough suppression after general anaesthesia, which includes retention of airway secretions, and infections. Cough can also be a warning sign of disease, and can cause the patient to seek medical attention, leading to diagnosis. When a cough is excessive and chronic, it can be detrimental to the patient with complications such as vomiting, rib fractures, urinary incontinence, syncope, muscle pain, tiredness, and depression (7;5).

The use of pleasanty coloured, flavoured and appropriately sweetened liquid dosage form know as cough syrup has been a mainstay practice in the treatment of disease conditions like cough in paediatric and geriatric age groups (2). Syrup is a concentrated sugar solution such as sucrose in water or other aqueous liquid, sometimes with a medicinal agent added; usually used as a flavoured vehicle for drugs. It is commonly expanded to include any liquid dosage form (oral suspension for example) in a sweet and viscid vehicle (8). However, these products face unique challenges in their production process. The mixing technology chosen for a given product will have a significant impact on its effectiveness and overall quality as the occurrence of microbial contamination has been well documented; contaminants range from true pathogens such as *Clostridium tetani* to opportunistic pathogens such as *Pseudomonas aeruginosa* (9).

Several reports have also been published describing clinical hazards that are attributed to microbiologically contaminated syrups (9). Since the manufacturing of syrups involves a series of unit operation such as milling, granulation, coating, tablet pressing and others, they may be exposed accidentally to microorganisms and this mishandling may result in a series of health hazard following ingestion of the highly contaminated drugs by patients whose immunity is already compromised by illness (9). Microbial infections are not only the result of the physical presence of microorganisms, but also their metabolites/toxins that become harmful even if they are found in minute quantities (9). The more serious problem arising from microbial contamination of drugs is the absence of obvious signs of spoilage, therefore there is a need to know the microbial content of all drug and medicines whether they be sterile or non-sterile (8,9). Microbial contamination of cough syrup can occur due to various reasons, such as poor manufacturing practices, inadequate sterilization of equipment, improper storage conditions, and contamination during the dispensing process. Cough syrup is typically a liquid formulation that contains water, sugars, and other ingredients that can provide an ideal environment for microbial growth.

Contamination of cough syrup with pathogenic microorganisms can pose a serious risk to human health. When individuals consume contaminated cough syrup, they can be exposed to harmful microorganisms that can cause a range of illnesses, including infections of the respiratory tract, gastrointestinal tract, and urinary tract. In addition, people who have weakened immune systems, such as the elderly, young children, and individuals with underlying medical conditions, are particularly vulnerable to the effects of microbial contamination in cough syrup. Moreover, cough syrup is often used to treat respiratory infections, which means that individuals who are already sick may be more susceptible to the negative effects of microbial contamination. The presence of viable and potentially pathogenic microorganisms in cough syrup can also compromise the efficacy of the medication, which can lead to inadequate treatment of the underlying illness Microbial contamination of cough syrup must be assessed to ensure the safety and efficacy of the product, meets regulatory requirements, maintain the reputation of the manufacturer, and protect the health of patients. Analyzing the microbial contaminants in cough syrup sold in Akungba Akoko will help to ensure the safety and effectiveness of the medicine and protect consumers from potential harm caused by contaminated products.

2.0 MATERIALS AND METHODS

Sample collection of Cough Syrup in Patent Medicine Shops, Akungba-Akoko Metropolis

Eight different cough syrups (Tutolin Expectorant-TX, Emzolin-EM, Exiplon Expectorant-EX, Coflin Expectorant-COF, Codolin Expectorant -COD, Cough 'N' cold- CNC, De Salom cof cough Expectorant-DES and Fitulin Expectorant- EX) were selected from patent medicine stores in Akungba-Akoko, Ondo, Nigeria.

Plate 1: *Picture of the cough syrups obtained from patent medicine store in Akingba-Akoko.*

Isolation, Identification and Characterization of isolates found in cough syrup

Bacteriological analysis of the cough syrup

Pour plate method of inoculation was used for the enumeration of bacteria; 0.5ml of the five -fold dilution of $10^{-3}$and 10^{-5}Cough syrup samples (inoculum) was put into sterile Petri dishes. MacConkey medium (55 grams of Agar into 1 liters), plate count medium (23.5 grams of agar into 1 liters) and blood medium (37 grams of Agar into 1 liters) were prepared according to the manufacturer prescription into sterile conical flask, corked with cotton and aluminum foil and then homogenized to dissolve. It was sterilized in an Autoclave at a temperature of 121°C for 15 minutes. After the sterilization, the medium was allowed to cool to about 45 °C. 20ml of the sterilized media were then poured into different sterile Petri dishes containing the 0.5ml of the inoculums aseptically and allowed to set. Then the plates were incubated at 37°C for 24hrs. After 24hrs, the cultural characteristics on the plates were studied and recorded. Resultant colonies were sub-cultured on fresh Nutrient agar and then incubated for 24hrs. Pure isolates were preserved on a double-strength nutrient agar slant for further studies (10).

Identification of Isolates from Cough Syrup in Patent Medicine Shops, Akungba-Akoko Metropolis

Microscopic examination and biochemical characteristics of isolates

To identify the pure isolate, both cultural and microscopic examinations were conducted. The initial identification of the isolates was based on the creamy pigmentation, round and slightly elevated shape, irregular and thread-like some of which are swarmy, with no distinct colony, and opaque cellular morphology characteristics. Furthermore, conventional identification of the isolates was carried out using various biochemical tests such as Catalase, Indole, Motility, Gram staining, Fermentation of sugars (Sucrose, Lactose, Dextrose), Urease, Hydrogen sulphide, Gas production, and oxidase tests.

Gram staining technique

A sterilized inoculating loop was used to make a smear of the culture onto a clean, grease-free slide. The slide was then labeled with each isolate code and heat fixed. The smear was flooded with crystal violet (primary stain) for 60 seconds and rinsed with water. Lugol's Iodine was applied to the slide as a mordant and left to stay for 1 minute. After that, it was rinsed with water and left for 30 seconds. 70% ethanol was used to decolorize the smear for 15 seconds, and it was immediately rinsed off in gently running tap water to remove the ethanol effect. The slide was then counterstained with safranin for 60 seconds, rinsed with water, and blot-dried. It was reported that the slides were viewed under the microscope using oil immersion (×100). It was observed that Gram-positive cells appeared purple since they retained the purple color of the primary stain (crystal violet) as they were not decolorized by alcohol. In contrast, Gram-negative cells appeared pink as the alcohol removed the crystal violet-iodine complex (11).

Biochemical characteristics of the isolates from Cough Syrup in Patent Medicine Shops, Akungba-Akoko Metropolis

Coagulase Test

A clean slide was prepared with a drop of normal saline on each end. They collected a colony of the test isolates using a sterile wire loop and mixed it with the saline drops on each end to create two suspensions.

Then a loopful of fresh human plasma was dropped on one end of the suspension and gently mixed it with a sterile wire loop, leaving the suspension on the other end as a control. Positive control was using *staphylococcus aureus* and observed for clumping. Finally, the results were recorded (11).

Catalase Test

This test is used to differentiate organism that have enzyme catalase, capable of decomposing hydrogen peroxide (H_2O_2). Two drops of hydrogen peroxide solution was dropped on a clean grease free glass slide, with an applicator stick, a colony from the stock isolate was picked and rocked on the slide with hydrogen peroxide solution, colonies that produced oxygen bubbles were recorded as being Catalase positive while those that did not produce bubbles were recorded as catalase negative The result was then recorded for each isolate (12).

Citrate test

Simmon's Citrate Agar is used to test the ability of an organism to utilize citrate as a source of energy. The medium contains citrate as the sole carbon source and Inorganic Ammonium Salts as the source of nitrogen. The agar was prepared by dissolving it with gentle heat and mixing until it dissolved. Then, 5 ml was dispensed into each tube and autoclaved at 121° C for 15 min. After cooling and slanting, the test organism was streaked with a light inoculum and incubated aerobically at 37° C for 5 days. The results were determined based on the color changes observed. Positive results were indicated by a change in color from green to blue or yellow, while negative results were indicated by the agar retaining the original green color. The experiment included uninoculated control tubes, and the results were recorded for analysis (13).

Indole Test

Motility, indole and urease broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. Colony was picked from the stocked culture slant and inoculated into the broth which was incubated for 24hours at 37^0C. After the incubation period, Kovac's reagent was added to the incubated broth culture, shaken gently and allowed to stand for 20 minutes and color change was observed. A red color change indicates positive result, while those that retained the color of the reagent indicated a negative result (14).

Motility test

Motility, indole and urease broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. Colony was picked from the stocked culture slant and inoculated into the broth which was incubated for 24hours at 37^0C. After the incubation period, a diffuse zone of growth flaring from the line of inoculation indicates a positive result and a restricted along the stab line indicates a negative result (15).

Oxidase test

Nutrient broth was prepared and autoclaved at 121^0C for 15 minutes; it was then allowed to cool. Colony was picked from the stocked culture slant and inoculated into the broth which was incubated for 24hours at 37^0C, after incubation, kovac's oxidase reagent was added to the culture, the colour change to purple indicates an oxidase positive and no colour change indicates an oxidase negative (16).

Urease test

Motility, indole and urease broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. Colony was picked from the stocked culture slant and inoculated into the broth which was incubated for 24hours at 37^0C. Urea solution was prepared and then added to the culture before incubation. After the incubation period, a colour change from yellow-orange to pink-red indicates a positive result and no colour indicates a negative reaction (17).

Fermentation of Dextrose, Lactose and sucrose and Hydrogen sulfide utilization and Gas production on tested samples.

Triple sugar iron agar was prepared and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. Colony was picked from the stocked culture slant and inoculated into the broth which was incubated for 24hours at 37^0C. After incubation, an alkaline or acid (red slant/yellow butt) reaction indicates dextrose fermentation only, an acid/acid (yellow slant/yellow butt) reaction indicates the fermentation of dextrose, lactose and sucrose, an alkaline/alkaline(red slant/ red butt) reaction indicates absence of carbohydrate fermentation results, blackening of the medium occurs in the presence of H_2, bubbles or cracks in the agar indicates the production of gas (formation of CO_2 and H_2) (18).

Fructose

3g of peptone powder was dissolved in 180ml of distilled water in appropriately labelled conical flask and 0.5g of phenol red was added. Ig of Fructose sugar was added into the conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop full of test organisms and incubated at 37 for maximum of 48 hours. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test (18).

Maltose test

A Carbohydrate fermentation test was carried out on maltose, on test isolates in an anaerobic condition. The nutrient broth was incorporated with any one of the above-mentioned carbohydrates along with a pH indicator phenol red which is red at pH 7 (neutral) and yellow at a pH of 6.8 (acidic) was used as the medium for carbohydrate fermentation test. This specified carbohydrate-containing media was prepared and inoculated with the isolates and Durham tubes were placed inside in an inverted position. The media was incubated in an anaerobic condition for 24 h at 30 °C. Fermentation of carbohydrates results in the formation of organic acids which changes the color of the media from pink to yellow, along with the liberation of gases which is entrapped in the Durham tubes (19).

Starch Hydrolysis Test

Nutrient agar was prepared and the isolates were inoculated onto the plates using a sterile inoculating loop with the streak method. The plates were then placed in an incubator at 37°C for 24 hours. After the incubation period, Gram's iodine was flooded onto the plates. Observations were made for a clear zone surrounding the test organisms as an indication of starch hydrolysis (20).

Molecular Identification of isolates from Cough Syrup in Patent Medicine Shops, Akungba-Akoko Metropolis

DNA extraction and PCR

DNA was extracted from the cultured bacteria in broth using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research) using the manufacturers procedures. Extracted DNA was then stored in -20 °C till PCR (Byadgi *et al.,* 2023). PCR sequencing preparation cocktail was prepared using (per reaction) 25 µlTaq 2X Master Mix | NEB, 4 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and made up to 42 µl with sterile distilled water to which 8µl DNA template was then added. Polymerase chain reaction (PCR) was carried out in a Gene Amp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds ; and a final termination at 72°C for 10 mins. And chill at 4oC.GEL (Osuntokun et al,.2024,Byadgi *et al.,* 2023).

Molecular Integrity

The integrity of the amplified gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells afterthe 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel (21,22)

Purification of Amplified Product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and kept at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µl of sterile distilled water and kept in -20oC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using anano drop of model 2000 from thermo scientific (23).

Molecular Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using the manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis (23,24).

Antibiotic Susceptibility Test of the Isolated Bacteria from Cough Syrup in Patent Medicine Shops, Akungba-Akoko Metropolis

Antibiotic susceptibility tests were carried out using the Kirby-Bauer disc diffusion method. Inoculum from bacterial isolates on slants was transferred into test tubes containing freshly sterilized nutrient broth and then incubated at 37°C for 15 hours to achieve a 0.5 McFarland standard. Mueller-Hinton agar was prepared and sterilized, and then dispensed into sterile Petri dishes. The plates were left to cool for approximately 15 minutes to allow them to gel and for excess surface moisture to be absorbed. The inoculum was then introduced onto the plates using the streaking method before applying the antibiotics-impregnated discs. The Gram-positive discs included Streptomycin, Ciprofloxacin, Rocephin, Amoxicillin, Ampiclox, Gentamycin, Pefloxacin, Erythromycin, Septrin, and Zinnacef. The Gram-negative discs consisted of Ciprofloxacin, Septrin, Ampicillin, Tarivid, Nalidix, Pefloxacin, Gentamycin, and Augmentin. The predetermined commercial Gram-negative and Gram-positive discs were aseptically applied to the surface of the well-labeled inoculated agar plates using sterile forceps, and they were then placed firmly by slightly pressing on the inoculated plates with the sterilized forceps to ensure complete contact with the agar. After 24 hours of incubation, each plate was examined for susceptibility to each antibiotic, which was indicated by a clear zone. The zone of inhibition was measured using a calibrated ruler held on the back of the inverted Petri plate and was recorded. Finally, the results were interpreted according to the Clinical Laboratory Standard Institute (10,24) guidelines.

Measurement of Death rate of the isolates using Ultra violet spectrophotometer

Growth dynamic refers to the rate at which cells of microorganism grow at a given time. This test was done to determine the rate of growth of the isolates as well as their killing time in due time. Colony was picked from the stocked culture slant and inoculated into nutrient broth which was incubated for 24hours at 37^0C. A loopful of organism was picked from the broth culture into nutrient broth in three sets which are set A and B respectively. Ultraviolet spectrophotometer was set at 620λ wavelength, warmed up for 15 minutes and then the control was first. For set A and B, the first reading was taken at zero hour, eighth hour and it continues after every 12 hours for 6 times. 0.5 ml of Ciprofloxacin was then put aseptically into the set B, at 24 hour to determine the killing time while set **A** was left to further check the growth rate of the microorganisms(25).

Measurement of Growth dynamic of the isolates using Ultra violet spectrophotometer

Growth dynamic test was done to determine the rate of growth of the isolates as well as the killing time after incorporation of antibiotic in due time. Colony of sample were picked from the stocked culture slant and inoculated into nutrient broth which was incubated for 24hours at 37^oC. A loopful of the each organism was picked from the broth culture into nutrient broth in two sets which are labeled as set A and B respectively.

The growth rate was measured using ultraviolet spectrophotometer; the spectrophotometer was set at 620λ

wavelength, warmed up for 15 minutes, calibrated before, the determination of the growth rate with sterilized nutrient broth spectrophotometer for set A. During the determination of the killing rate graded amount of Ciprofloxacin were incorporated into the organisms in set B as well as the sterilized nutrient broth which was used to calibrate the spectrophotometer after taken first 3 readings. The first was reading was taken at zero hour, eighth hour and it continues after every 12 hours for 6 times. At the 4th reading for set B, which is the 48th hour of set B, 1ml of 0.5mg/ml of Ciprofloxacin was added to determine up the rate of kill (26).

3.0 RESULTS

Table 1 shows the production date, expiration date, Company/location of production, and the total number of cough syrup used for this study. A total of eight (8) different cough syrups were obtained from patent sellers in Akungba-Akoko. It was observed that all the cough syrups used in this study were unexpired.

Table 2 Shows the dilution factors of the cough syrup isolates and their colony count on MacConkey agar, blood agar, and plate count agar. In this table, it was observed that 20 plates were recovered and each plate contains inoculum in serial dilutions, the highest dilution factor is the 10^3 and the lowest dilution factor is the 10^4. FIT A, EM A, EM C, EX1 B, EX1 D, COF A, COF C, CNC A, DES B, TX A and COD A have dilution factor 10^3, while COD B, TX B, DES A, CNC B, COF B, EX1 C, EX1 A, EM B and FIT B have dilution factor 10^4. DES A has the highest colony count which is 75 while, EM C has the lowest colony count which is 10^5.

TABLE 3 shows the morphological characteristics of cough syrup isolates on nutrient agar. In this table, it was observed that FIT A, EM C, EX1 A and EX1 B, were irregular in shape while, the rest of the isolate were circular in shape except DES A, TX A and COD A which were filamentous and rhizoid respectively. COF A, COF B and COD A were white in colour while, the rest of the isolates were cream in colour. FIT A, EM C, EX1 A, DES B and DES A have convex papilate elevation while, the rest of the isolates have convex elevation. EX1 A, EX1 B and DES A have a lobate edge while, the rest of the isolate has an entire edge except EM C and DES B that has tentate and fimbrate edge. It was shown in this table that FIT B, EM A, EM B, EX1 C, EX1 D, CNC A, CNC B, TX B and COD B has the same morphological characteristic. COF A and COF B also has the same morphological characteristics.

Table 1: The List of Cough Syrups Obtained From Patent Medicine's Store, Production Date, Expire Date and Company/ Location of Production

COUGH SYRUP	PRODUCTION DATE	EXPIRE DATE	COMPANY/ LOCATION
Exiplon-expectorant (EX)	07-2021	06-2024	Unique Pharmaceuticals Ltd. Abeokuta, Ogun State.
Cof 'N' Cold (COF)	03-2021	03-2024	Peace Standard Pharma- ceutical Ind. Ltd. Kwara State
Coflin-expectorant (COF)	07-2021	07-2023	Vitabiotics (Nig.) Ltd, Ikeja, Lagos.
Emzolyn (EM)	10-2019	10-2022	Mzor Pharmaceutical Ltd., Isolo Lagos state
De-shalom cof cough expectorant (DES)	02-2022	01-2024	De-shalom Pharmaceutical lab Ltd., Ilesa, Osun State
Tutolin expectorant (TX)	07-2021	07-2023	Tuyil Pharmaceutical, Ltd., Ilorin, Kwara state
Codolin expectorant (COD)	05-2021	05-2024	Tuyil Pharmaceutical, Ltd., Ilorin, Kwara state.
Fitulin expectorant (FIT)	02-2022	01-2022	Fidson healthcare plc. Sango-Ota, Ogun State.

Table 2: Dilution Factors and Colony Count of Cough syrup bacteria Isolates on MacConkey Agar, plate count agar and Blood Agar

Sample code	Dilution factor	Number of colony
FIT A	10^3	60
FIT B	10^4	40
EM A	10^3	9
EM B	10^4	21
EM C	10^3	5
EX1 A	10^4	35
EX1 B	10^3	17
EX1 C	10^4	12
EX1 D	10^3	34
COF A	10^3	20
COF B	10^4	65
COF C	10^3	40
CNC A	10^3	60
CNC B	10^4	40
DES B	10^3	51
DES A	10^4	72
TX A	10^3	12
TX B	10^4	8
COD A	10^3	14
COD B	10^4	15

KEY: Exiplon-expectorant (EX), Cof 'N' Cold (COF), Coflin-expectorant (COF), Emzolyn (EM), De-shalom cof cough expectorant (DES), Tutolin expectorant (TX), Codolin expectorant (COD), Fitulin expectorant (FIT)

Table 3: Sample Code, Morphological Characteristics of cough syrup bacteria Isolates on Nutrient Agar

Isolates	shape	Colour	Elevation	Edge
FIT A	Irregulalar	Cream	Convex papilate	Fimbrate
FIT B	Circular	Cream	Convex	Entire
EM A	Circular	Cream	Convex	Entire
EM B	Circular	Cream	Convex	Entire
EM C	Irregulalar	Cream	Convex papilate	Tentate
EX1 A	Irregulalar	Cream	Convex papilate	Lobate
EX1 B	Irregulalar	Cream	Convex	Lobate
EX1 C	Circular	Cream	Convex	Entire
EX1 D	Circular	Cream	Convex	Entire
COF A	Circular	White	Convex	Entire
COF B	Circular	White	Convex	Entire
COF C	Rhizoid	Cream	Convex	Entire
CNC A	Circular	Cream	Convex	Entire
CNC B	Circular	Cream	Convex	Entire
DES B	Rhizoid	Cream	Convex papilate	Fimbrate
DES A	Filamentous	Cream	Convex papilate	Lobate
TX A	Rhizoid	Cream	Convex	Rhizoid
TX B	Circular	Cream	Convex	Entire
COD A	Rhizoid	White	Convex	Entire
COD B	Circular	Cream	Convex	Entire

KEY: Exiplon-expectorant (EX), Cof 'N' Cold (COF), Coflin-expectorant (COF), Emzolyn (EM), De-shalom cof cough expectorant (DES), Tutolin expectorant (TX), Codolin expectorant (COD), Fitulin expectorant (FIT)

Table 4: Shows the result of Gram staining and the microscopic examination of the cough syrup isolates. It was observed in this table that EM B, CNC A, CNC B and DES B were positive to Gram staining while the rest of the isolates were negative to Gram staining. This table also shows the shapes of the recovered isolates, FIT A, EM A, COF B and CNC A has a cocci shape under the microscope while, FIT B, TX B, COF A, CNC B, DES B, TX A and COD B havs long rod shape. EM B, EM C, EX1 A, EX1 B, EX1 C, EX1 D, COF C, DES A and COD all havs a short rod shape.

Table 5: The table shows result of biochemical tests and sugar fermentation tests done for the cough syrup isolates. In this table it was shown that Fit A was negative to the Catalase test and the rest of the isolates were positive to Catalase test. It was also shown in this table that Fit A, Fit B and EM A were negative to Oxidase test while the rest of the isolates were positive to it. Fit A, Fit B, EM A, EX C, COF B, COF C and TX A were negative to the Coagulase test while, the rest of the isolates were positive to Coagulase test. DES B, DES A, CNC A, COF C, COF B, COF A and Fit A. were negative to Citrate test while, the rest of the isolates were positive to the Citrate test. Fit B, EM A, EM B, DES A and TX B were negative to starch hydrolysis test while the rest of the isolates were positive to starch hydrolysis test. Fit A and EM A were negative to the motility test while, the rest of the isolates were positive to motility test. All the isolates were positive to Indole. Fit A and EM B were negative to Urease test while, the rest of the isolates were positive to it. All the isolates were positive to glucose, fructose and maltose fermentation. Fit A and Em A are positive to lactose sugar fermentation while the rest of the isolates were negative to it. COF A, EM C, EM B, COF B and DES A were negative to Hydrogen sulfide utilization, while the rest were positive to it. Ex 1 was negative to sucrose fermentation while the rest of the isolates were positive to it. EX C and EX 1 were negative to dextrose sugar fermentation while, the rest were positive to dextrose sugar fermentation.

Table 4: Gram Stain and Microscopic Examination on the Recovered Cough Syrup Isolates

Isolates	Gram stain	Shape
FIT A	-	Cocci
FIT B	-	Long rods
EM A	-	Cocci
EM B	+	Short Rods
EM C	-	Short Rods
EX1 A	-	Short rods
EX1 B	-	Short rods
EX1 C	-	Short Rods
EX1 D	-	Short rods
COF A	-	Long rods
COF B	-	Cocci
COF C	-	Short rods
CNC A	+	Cocci
CNC B	+	Long rods
DES B	+	Long rods
DES A	-	Short Rods
TX A	-	Long rods
TX B	-	Long Rod
COD A	-	Short Rod
COD B	-	Long rods

KEY: Exiplon-expectorant (EX), Cof 'N' Cold (COF), Coflin-expectorant (COF), Emzolyn (EM), De-shalom cof cough expectorant (DES), Tutolin expectorant (TX), Codolin expectorant (COD), Fitulin expectorant (FIT)

Table 5.1: Preliminary Biochemical Tests carried on Cough syrup bacteria Isolates Bacteria from Cough Syrup in Patent Medicine Shops, Akungba-Akoko Metropolis

Isolates	Catalase test	Oxidase test	Coagulase test	Citrate test	Motility test	Indole test	Urease test	Lactose	Dextrose	Sucrose	H_2S production	Glucose	Fructose	maltose	starch hydrolysis
COF B	+	+	-	-	+	-	+	-	+	+++	-	+++	+	+	+
COF C	+	+	-	-	++	-	+	-	++	++	++	++	++	+	+
CNC A	+	+	+	-	+	-	++	-	+	++g	++	+++	++	++	+
CNC B	+	+	+	+	+	-	+	-	++	+	+++	++	+++	+	+
DES A	+	+	+	-	++	-	+	-	+	+	-	++	++	++	-
DES B	+	+	+	-	+	-	+	-	++	++	++	+++	+	-	+
TX A	+	+	-	+	+	-	++	-	++	+++	+++	++	+	+++	+
TX B	+	+	+	+	+	-	+	-	++	++	+++	+++	+	+++	-
COD A	+	+	+	+	++	-	+	-	++	++g	++	+++	+	+	+
COD B	+	+	+	+	++	-	+	-	++	++	++	+++	+++g	+++	+

Key- positive +, negative –, strong positive ++, very strong positive+++

Table 5.2: Preliminary Biochemical Tests carried on cough syrup Isolates

Isolates	Catalase test	Oxidase test	Coagulase test	Citrate test	Motility test	Indole test	Urease test	Lactose	dextrose	Sucrose	H_2S production	Glucose	Fructose	Maltose	starch hydrolysis
Fit A	-	-	-	-	-	-	-	+	++	+++	++	+++	++	+++	+
fit B	+	-	-	+	+	-	+	-	+++	++	+++	++	++	++	-
EM A	+	-	-	+	-	-	+	+	+++	+++	+++	++	++	+	-
EM B	+	+	+	+	+	-	-	-	+	+++	-	+	++	+	-
EM C	+	+	+	+	++	-	+	-	++	+++g	-	+++g	+++g	+++	+
EX1	+	+	+	+	+	-	+	-	-	-	+++	+++g	++	+++	+
EX 2	+	+	+	+	++	-	+	-	+++	++	+++	+++g	++g	++	+
EX C	+	+	-	+	++	-	+	-	-	+++	++	++	+++	+++	+
EX D	+	+	+	+	+	-	++	-	+++	++	++	+++	++g	++	+
COFA	+	+	+	-	+	-	++	-	++	++	-	+++	+	+++	+

Key – positive +, negative -, strong positive ++, very strong positive +++
Exiplon-expectorant (EX), Cof 'N' Cold (COF), Coflin-expectorant (COF), Emzolyn (EM), De-shalom cof cough expectorant (DES), Tutolin expectorant (TX), Codolin expectorant (COD), Fitulin expectorant (FIT)

Table 5.3: List of Cough syrup isolates characterize using Bergey's Manual of Determinative Bacteriology

Isolates	Probable organism
FIT A	*Streptococcus pneumonia*
FIT B	*Proteus mirabilis*
EM A	*Staphylococcus aureus*
EM B	*Bacillus subtilis*
EM C	*Proteus mirabilis*
EX1 A	*Citrobacter freundii*
EX1 B	*Proteus mirabilis*
EX1 C	*Enterobacter sp.*
EX1 D	*klebsiella sp.*
COF A	*Enterobacter sp.*
COF B	*Salmonella sp.*
COF C	*Proteus sp.*
CNC A	*Streptococcus sp.*
CNC B	*Bacillus subtilis*
DES B	*Staphylococcus aureus*
DES A	*Bacillus subtilis*
TX A	*Enterobacter sp.*
TX B	*Klebsiella sp.*
COD A	*Salmonella sp.*
COD B	*Salmonella sp.*

Fig 1: Sample ID "Fit A (1)" has a max score and total score of 2724, a query cover of 100%, an E value of 0, and a percent identity of 99.66%. The scientific name of the sample was identified as *Streptococcus pneumonia.* Similarly, sample ID "DES A (15)" has a max score and total score of 2719, a query cover of 99%, an E value of 0, and a percent identity of 99.60%. The scientific name of this sample was identified as *Staphylococcus aureus*

Fig 2: Shows the antibiotic susceptibility test of the identified Gram negative organisms from cough syrup. In this table, it was observed *Streptococcus pneumonia* (Fit A), was sensitive to tarivid, pefloxacin, gentamycin, and ciprofloxacin, with intermediate sensitivity to augumentin. It was also resistant to nalidixic acid, septrin, gentamycin, and ampicilin. *Proteus mirabilis* (Fit B) was sensitive to tarivid, pefloxacin, ciprofloxacin, and septrin. However, it was resistant to nalidixic acid, gentamycin, and ampicilin. *Proteus mirabilis* (EM C) was sensitive to tarivid, pefloxacin, and ciprofloxacin, but resistant to nalidixic acid, gentamycin, and ampicilin. *Citrobacter Freundii* (EX1) was sensitive to all antibiotics except nalidixic acid, which it showed intermediate sensitivity to. Proteus mirabilis (EX2) was sensitive to tarivid, pefloxacin, and ciprofloxacin but resistant to nalidixic acid, gentamycin, and ampicilin. *Enterobacter* sp. (EX C) was sensitive to all antibiotics except ampicilin, which it showed intermediate sensitivity to, and ceporex, which it was resistant to. *Klebsiella* sp. (EX D) was sensitive to tarivid, pefloxacin, ciprofloxacin, and septrin but resistant to nalidixic acid, gentamycin, and ampicilin. *Enterobacter* sp. (COFA) was sensitive to tarivid, pefloxacin, and ciprofloxacin but resistant to gentamycin. *Salmonella* sp. identified as COF B was sensitive to tarivid, pefloxacin, and ciprofloxacin but resistant to gentamycin. *Proteus* sp. identified as COF C was sensitive to tarivid, gentamycin, and AU but resistant to nalidixic acid, pefloxacin, and septrin. *Streptococcus* sp. (DES B) was sensitive to tarivid, pefloxacin, gentamycin, ciprofloxacin, and septrin but resistant to nalidixic acid and ampicilin. *Enterobacter* sp. (TX A) was sensitive to tarivid, PEF, gentamycin, ciprofloxacin, and septrin but showed intermediate sensitivity to augumentin. It was also resistant to ampicilin. *Klebsiella* sp. (TX B) was sensitive to all antibiotics except ampicilin, which it showed intermediate sensitivity to. *Salmonella* sp. (COD A) was sensitive to tarivid, gentamycin, and ciprofloxacin but resistant to ampicilin and showed intermediate sensitivity to PEF and Augumentin. *Salmonella* sp. (COD B) was sensitive to tarivid, PEF, ciprofloxacin, and septrin, but resistant to nalidixic acid and showed intermediate sensitivity to gentamycin.

Fig. 1: Agarose gel showing the positive amplification of cough syrup isolates (FIT A -1 and DES A-15). Band size of 1500bp indicates positive amplification.

: Max Score, Total Score, Query Cover, E value, Per. Ident and Accession

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Fit A (1)	*Streptococcus pneumonia*	2724	2724	100%	0	99.66%	
DES A (15)	*Staphylococcus aureus*	2719	2719	99%	0	99.60%	

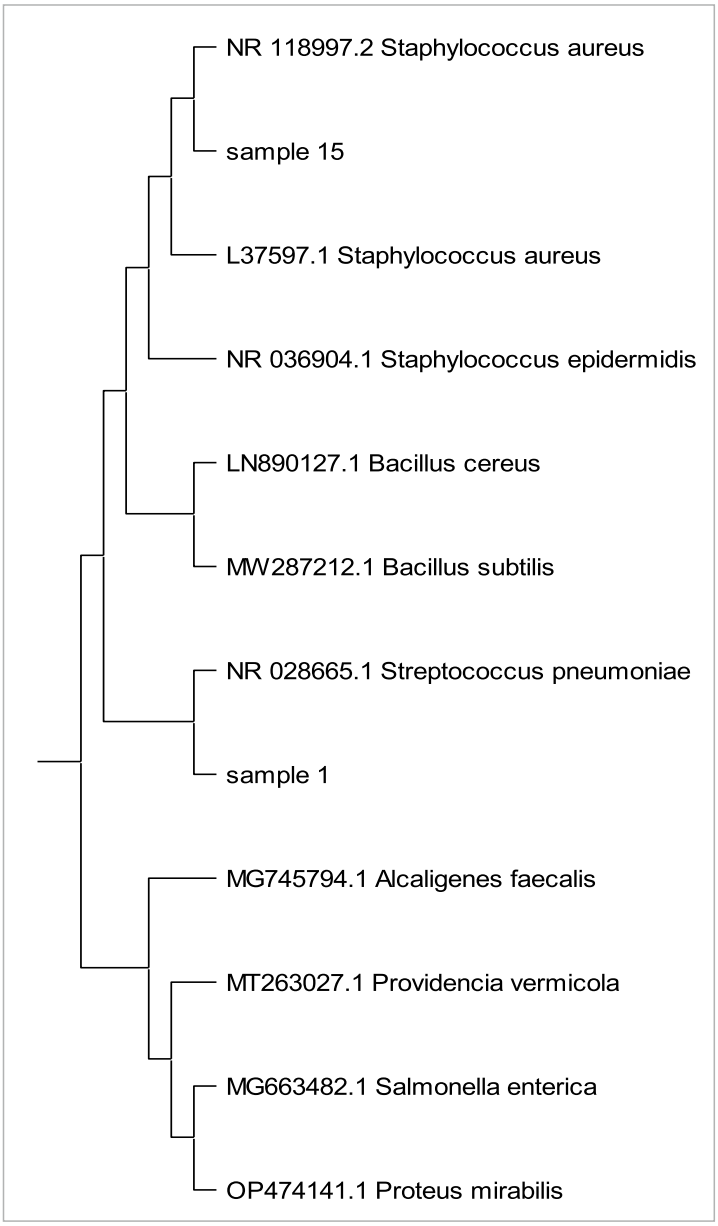
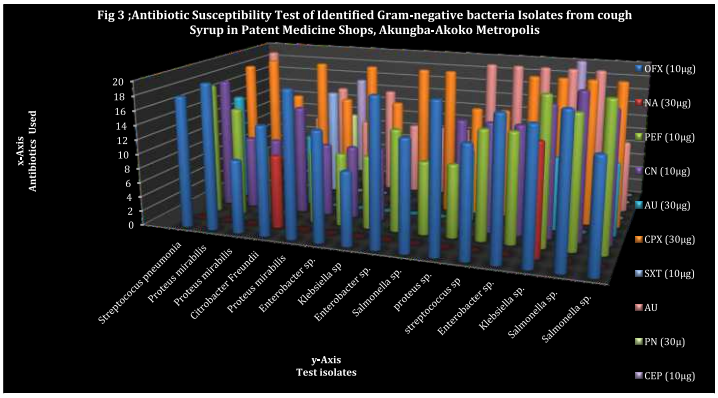


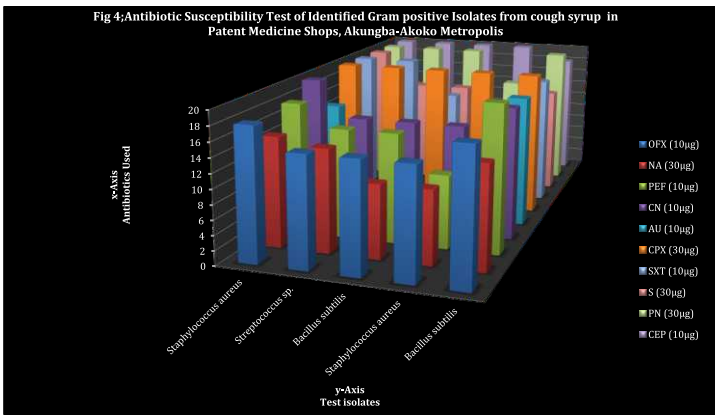
Fig 2. phylogenetic tree of the isolated bacteria from cough syrub (Sample 1 and sample 15)

Fig 3: Shows the antibiotic susceptibility test of the identified Gram positive organisms from cough syrup. The results indicate that the antibiotics Tarivid (OFX), Pefloxacin (PEF), Ciprofloxacin (CPX), Septrin (SXT), Ampicillin (PN), and Ceporex (CEP) were effective against all the isolates tested. On the other hand, the antibiotics Nalidixic acid (NA) and Gentamycin (CN) showed intermediate susceptibility against some isolates, while Streptomycin (S) showed intermediate susceptibility against one isolate. The table also shows that the *Staphylococcus aureus* isolates have a sensitivity of 18 mm to Tarivid (OFX), 15 mm to Nalidixic acid (NA), 18 mm to Pefloxacin (PEF), 20 mm to Gentamycin (CN), 15 mm to Augmentin (AU), 20 mm to Ciprofloxacin (CPX), 20 mm to Septrin (SXT), 20 mm to Streptomycin (S), 20 mm to Ampicillin (PN), and 20 mm to Ceporex (CEP). Similarly, the *Streptococcus* sp. isolates are sensitive to Tarivid (OFX) with a sensitivity of 15 mm, 14 mm to Nalidixic acid (NA), 15 mm to Pefloxacin (PEF), 15 mm to Gentamycin (CN), 20 mm to Ciprofloxacin (CPX), 20 mm to Septrin (SXT), 15 mm to Streptomycin (S), 20 mm to Ampicillin (PN), and 20 mm to Ceporex (CEP). *Bacillus subtilis* isolates showed sensitivity to Tarivid (OFX) with a sensitivity of 18 mm, 14 mm to Nalidixic acid (NA), 20 mm to Pefloxacin (PEF), 18 mm to Gentamycin (CN), 18 mm to Augmentin (AU), 20 mm to Ciprofloxacin (CPX), 18 mm to Septrin (SXT), 15 mm to Streptomycin (S), 20 mm to Ampicillin (PN), and 18 mm to Ceporex (CEP).

Fig 4: Shows the antibiotic susceptibility test of the identified Gram-positive organisms from cough syrup. The results indicate that the antibiotics Tarivid (OFX), Pefloxacin (PEF), Ciprofloxacin (CPX), Septrin (SXT), Ampicillin (PN), and Ceporex (CEP) were effective against all the isolates tested. On the other hand, the antibiotics Nalidixic acid (NA) and Gentamycin (CN) showed intermediate susceptibility against some isolates, while Streptomycin (S) showed intermediate susceptibility against one isolate. The table also shows that the *Staphylococcus aureus* isolates have a sensitivity of 18 mm to Tarivid (OFX), 15 mm to Nalidixic acid (NA), 18 mm to Pefloxacin (PEF), 20 mm to Gentamycin (CN), 15 mm to Augmentin (AU), 20 mm to Ciprofloxacin (CPX), 20 mm to Septrin (SXT), 20 mm to Streptomycin (S), 20 mm to Ampicillin (PN), and 20 mm to Ceporex (CEP). Similarly, the *Streptococcus* sp. isolates are sensitive to Tarivid (OFX) with a sensitivity of 15 mm, 14 mm to Nalidixic acid (NA), 15 mm to Pefloxacin (PEF), 15 mm to Gentamycin (CN), 20 mm to Ciprofloxacin (CPX), 20 mm to Septrin (SXT), 15 mm to Streptomycin (S), 20 mm to Ampicillin (PN), and 20 mm to Ceporex (CEP). *Bacillus subtilis* isolates showed sensitivity to Tarivid (OFX) with a sensitivity of 18 mm, 14 mm to Nalidixic acid (NA), 20 mm to Pefloxacin (PEF), 18 mm to Gentamycin (CN), 18 mm to Augmentin (AU), 20 mm to Ciprofloxacin (CPX), 18 mm to Septrin (SXT), 15 mm to Streptomycin (S), 20 mm to Ampicillin (PN), and 18 mm to Ceporex (CEP).



KEY: ≥ 16 = Sensitive, ≤ 14 = Resistant 15 = Intermediate S- Streptomycin, PN- Ampicilin, CEP- Ceporex, OFX- Tarivid, NA- Nalidixic acid, PEF- Reflacine, CN- Gentamycin, AU- Augmentin, CPX- Ciprofloxacin, SXT- Septrin UNIT: Milimetre (mm)



KEY: ≥ 16 = Sensitive, ≤ 14 = Resistant 15 = Intermediate S- Streptomycin, PN- Ampicilin, CEP- Ceporex, OFX- Tarivid, NA- Nalidixic acid, PEF- pefloxacin, CN- Gentamycin, AU- Augmentin, CPX- Ciprofloxacin, SXT- Septrin UNIT: Milimetre (mm)

Fig 5; Shows the growth dynamic of bacteria isolates gotten from cough syrup using ultraviolet Spectrophotometer with 620λ. In this table, it was observed that at 0 hour, *Bacillus subtilis* has the highest growth rate of 0.052λ while, *Proteus mirabilis* has the lowest growth rate of 0.161λ. At 64th hour *Enterobacter sp.* has the lowest death rate of 0.397λ while, *staphylococcus aureus* and *Salmonella sp.* has the highest death rate of 0.125λ

Fig 6 ; Shows the growth dynamic of bacteria isolates gotten from cough syrup using ultraviolet Spectrophotometer with 620λ. In this table, it was observed that at 0 hour, *Klebsiella ornithinolytica* has the highest growth rate of 0.205λ and *Escherichia coli* has the lowest growth rate of 0.039λ. At 64th hour *Enterobacter agglomerans* has the lowest death rate of 0.481λ and *Klebsiella ornithinolytica* has the highest death rate of 0.139λ

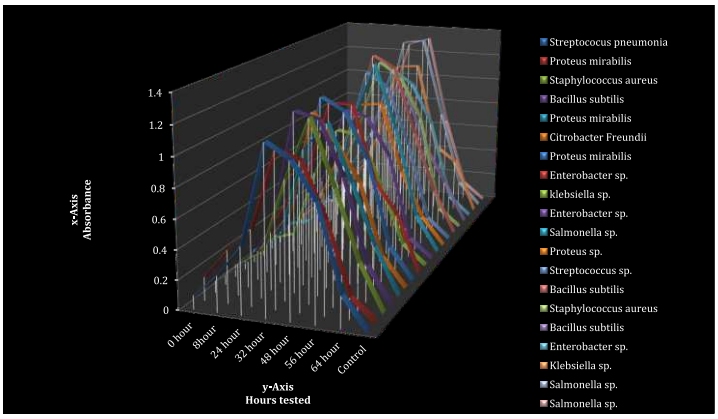


Fig 5: Growth Dynamic of Bacterial isolates from cough syrup Using Ultraviolet Spectrophotometer with Wavelength 620λ

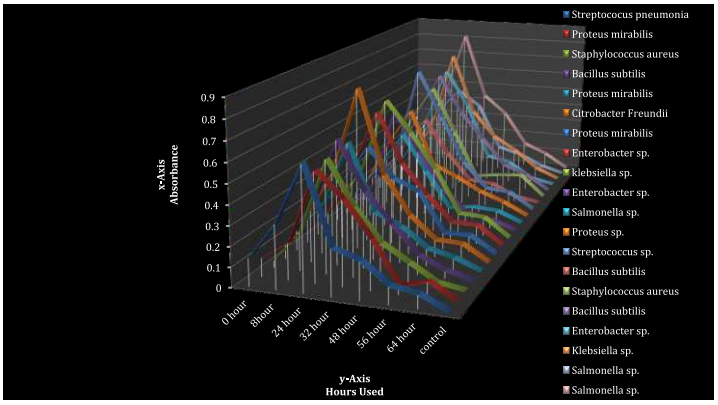


Fig 6: Growth Dynamic and Killing Time of Bacterial Isolates from cough syrup with Addition of Ciprofloxacin Antibiotic at the 24th Hour Using Ultraviolet Spectrophotometer with Wavelength 620

DISCUSSION

The aim of this study is, to isolate, identify and characterize microbial analysis & biohazards signatures of cough syrup isolates from patent medicine shop in Akungba-Akoko metropolis and determined the antibiotics pattern of the isolates from cough syrup. In the cough syrup and other conventional medicine, the microbial load should have been reduce to the nearest decimal point or absolute zero, but during this study , it weas observed that the number of microbe is very alarming and absolute measures and precaution needs to be taken into cognizant . otherwise, what is meant for cure will absolute become poisoning especially for the little kids, drastic measure need to be put in place holistically Therefore, regardless of pharmaceutical dosage form and route of administration, the microbiological content in non sterile pharmaceutical preparations must conform to the microbiological purity criteria set out in standard (2).

In this study, eight (8) cough syrups namely (Tutolin Expectorant-TX, Emzolin-EM, Exiplon Expectorant-EX, Coflin Expectorant-COF, Codolin Expectorant -COD, Cough 'N' cold-CNC, De Salom cof cough Expectorant-DES and Fitulin Expectorant- EX) were purchased from patent medicine stores in Akungba-Akoko metropolis were analyzed. Different percentages of organisms were isolated using microbiological laboratory techniques like macroscopic examination and microscopic examinations. Preliminary biochemical tests, sugar fermentation and molecular identification were done, to further identify the bacterial organisms. The antibiotics susceptibility patterns of the isolated bacteria were determined using Kirby-Bauer method. The growth dynamic and death rate of the isolates were also determined with the aid of Ultraviolet-visible spectrophotometer. it was observed from the result obtained from this study demonstrated that microorganisms are widespread in the selected cough syrup, it is a great surprise, that the harzard analysis and critical control point were not adequately followed , this can be a great threat and menace to the Akugba Akoko metropolis and Nigeria as a whole . adequate measures need to be taken to reduce this threat and children mortality. .

The results of the study confirm the presence of viable and potentially pathogenic microorganisms in all the cough syrups examined during this study, which were obtained from patent medicine stores in Akungba-Akoko, Ondo State, The microorganisms isolated, 75% were Gram-negative, while the remaining 25% were Gram-positive which includes *Staphylococcus aureus* (2), *Bacillus* spp (2), *Proteus* spp (4), *Citrobacter freundii* (1), *Enterobacter* spp (3)., *klebsiella* spp.(1) and *Salmonella* spp.(3), *Streptococcus* spp.(2). These findings are consistent with the study by (27) on microbial contamination of non-sterile pharmaceuticals in public hospital settings, and (2) on bacteriological screening of paediatric cough syrups marketed within Port Harcourt Metropolis, South-South Nigeria. Microbial contamination of pharmaceutical products has been known to affect the desirable characteristics of the formulation such as the physicochemical and therapeutic properties (28). This can occur during production, storage, from water, other raw materials, environment, excipients and personnel.

Moreover, heavy contamination by *Staphylococcus aureus* was expected, it is part of the normal body flora just like *Klebsiella. Pneumonia, Enterobacter* spp. are found in human intestines, but they have the propensity of causing skin infections, pneumonia, meningitis, destructive changes to the lungs and bacteremia, this may be due to the packaging process and handler of pharmaceutical processes, Similarly, the presence of *Bacillus subtillis* can be traced to the fact that it is abundant spore former in air and water used for production and poor quality/ assurance control facilities, and its presence in cough syrup can also be attributed to poor manufacturing practices, inadequate storage conditions, or contamination during the handling and dispensing of the product (29).

The colony count is another important parameter that indicates the number of viable microorganisms in a sample. The higher the colony count, the higher the concentration of microorganisms (30). In this study, the results in Table 1 show that the colony counts ranged from 5 to 72, which indicates a wide variation in the microbial load of the cough syrup isolates. These findings are consistent with the study by (31) on Assessment of microbial contamination in pediatric oral liquid formulations marketed in Katsina State, Nigeria, where the

occurrence of microbial contamination of the tested non-sterile pharmaceutical oral drug formulations was 65.7%.

Regarding the antibiotic susceptibility tests, the bacteria isolated showed varying degrees of sensitivity and resistance to different antibiotics. 62% of the Gram-negative microorganisms identified were resistance to the antibiotics used, while 20% of the Gram positive microorganisms were resistant to the antibiotics used. These antibiotic susceptibility patterns suggest that the Gram-negative isolates identified from cough syrup are resistant to some antibiotics commonly used to treat infections. This finding emphasizes the importance of conducting routine microbial analyses of cough syrup and other medications to detect and prevent the spread of antibiotic-resistant pathogens (32).

This study shows that some pharmaceutical companies in Nigeria do not always maintain sterile conditions, which can lead to contamination of their products. (33). Non-sterile syrups can be significantly harmful to patients who are already sick. The contamination of microorganisms in cough syrups can occur due to improper procedures during repackaging into smaller packs, handling of the handlers, poor hygienic conditions, and dispensing of syrups. Therefore, it is necessary to examine the efficacy and potency of commonly used cough syrups.

In developed countries, it is important for people to follow rules and regulations related to working conditions and hygiene. For example, pharmacies in Nigeria should follow a hygienic environment during the manufacturing and packaging of pharmaceutical products (34,35). This would require a strong quality control program and compliance with aseptic techniques during the preparation and packaging of pharmaceuticals, as well as education on personal hygiene for the personnel involved. By following these measures, the risk of microbial, physical, or other types of contamination could be reduced. Therefore, it is crucial to maintain the best hygiene conditions during the manufacturing of these products (36,36,37).

4.2 CONCLUSION

In conclusion, the findings of this study have significant implications for public health. The quality and safety of cough syrup products can directly impact the health and well-being of consumers. The results of this study demonstrate the need for greater regulation and oversight of the production and distribution of cough syrup products in patent shops to ensure their safety and effectiveness for their intended use. Additionally, this study highlights the importance of continuous quality assessment for pharmaceutical products, especially for non-sterile liquid dosage forms like cough syrup, multivitamin syrup, antacid syrup, and others, to guarantee their safety and efficacy for consumers.

4.3 RECOMMENDATION

Based on the results of the study on molecular identification and ultraviolet spectrophotometry signature of cough syrup isolates from patent medicine shops in Akungba-Akoko metropolis, I would recommend the following:

1. Stringent measures should be taken during the manufacturing, packaging, and distribution of cough syrups. Effective post-marketing surveillance should also be implemented. Consumers should be made aware of proper handling and storage procedures to maintain the microbiological quality of their medication.

2. Regular monitoring and evaluation of the quality of cough syrups sold in patent medicine shops in the metropolis should be conducted. This will ensure that the cough syrups meet required standards and are safe for human consumption.

3. A proper regulatory framework should be put in place to ensure that patent medicine shops in the metropolis comply with the standards set by the National Agency for Food and Drug Administration and Control (NAFDAC) and other relevant regulatory agencies.

4. The use of molecular identification and ultraviolet spectrophotometry should be encouraged as a means of identifying and characterizing pathogens in cough syrups and other pharmaceutical products sold in patent medicine shops. This will help to ensure that only safe and effective cough syrups are sold to the public.

Funding - This work was self supported

Acknowledgements -The authors wish to express appreciation to all the technical staff of the laboratory unit of the Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Competing Interests -Authors have declared that no competing interests exist

Dr Oludare temitope osuntokun- https://orcid.org/0000-0002-3954-6778, **Web of science Your ResearcherID** -L-4314-2016

References

1. Kaushik, A., Chauhan, V. and Sudha, D. (2016). Formulation and evaluation of herbal cough syrup. *European Journal of pharmaceutical and medical Research*, *3*(5): 517-522.

2. Ibezim, C., Nwosu, C. and Ogbu, H. (2018). Bacteriological Screening of Paediatric Cough Syrups Marketed Within Port Harcourt Metropolis, South-South Nigeria. *International Journal of Pharmaceutical Sciences and Drug Research*,*10*(2): 65-70.

3. De Blasio, F., Dicpinigaitis, P.V., Rubin, B.K., De Danieli, G., Lanata, L. and Zanasi A (2012). An observational study on cough in children: epidemiology, impact on quality of sleep and treatment outcome. *Bio Med Central*, 8(1):1-6

4. Coutier-Marie, L., Ioan, I., Bonabel, C., Demoulin, B., Leblanc, A. L., Debitu, L. and Demoulin-Alexikova, S. (2017). Maturation of airway defensive reflexes is related to development of feeding behavior during growth in rabbits. *Frontiers in Physiology*, *8*, 64.

5. Chung, K. F. and Pavord, I. D. (2008). Prevalence, pathogenesis, and causes of chronic cough. *The Lancet*,*371*(9621): 1364-1374.

6. Elghamoudi, D. (2018). *Objective Cough Monitoring in Children*. The University of Manchester (United Kingdom).

7. Michaudet, C. and Malaty, J. (2017). Chronic cough: evaluation and management. *American family physician*, *96*(9): 575-580

8. Studdert, V., Gay, C. and Hinchcliff, K. W. (2020). *Saunders comprehensive veterinary dictionary*. Elsevier Health Sciences.

9. Imarenezor, E. P. K., Abhadionmhen, O. A., Shinggu, P. P., Briska, J., George, O. S. and Danya, S. (2021). Bacterial contamination of retailed syrups traded in Wukari, North East, Nigeria. *International Journal of Biological and Pharmaceutical Sciences Archive*, 2021, 02(01): 117–125

10. Clinical and Laboratory Standards Institute. (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. *Approved standard M7-A7*, *26*.

11. Fawole, M.O. and Oso. B.A. (2007). Laboratory manual of Microbiology. Revised Edition spectrum books Ltd. Ibadan.;127

12. Izna, D. N., Misra, R. N., Mirza, S. B. and Das, N. (2019). Isolation of *Staphylococcus aureus* in Purulent Infective Conditions with Special Reference to MRSA.

13. Cappuccino, J.G., and Sherman, N. (2005). Microbiology A Laboratory Manual, 7th Edition Benjamin Cummings:Pearson Education, Inc. New York.

14. MacWilliams, M. P. (2012). Indole test protocol. *American Society for Microbiology, Washington, DC*. pg. 1-9.

15. Shields, P. and Cathcart, L. (2011). Motility test medium protocol. *American society for microbiology*, pg. 1-6.

16. Vivien, R., Holzmann, M., Werner, I., Pawlowski, J., Lafont, M. and Ferrari, B. J. (2017). Cytochrome c oxidase barcodes for aquatic oligochaete identification: development of a Swiss reference database. *PeerJ*, *5*, e4122

17. Brink, B. (2010). Urease test protocol. *American society for microbiology*, pg.1-7.

18. Randall, N. A., Nance, C., Huggins, C., Huggins, J. and Bolyard, M. (2017). Identification of an Alternative to Proteus vulgaris as a Laboratory Standard for Hydrogen Sulfide Production. *Journal of Microbiology and Biology Education*, *18*(1): 18-1.

19. Agrahari S, Wadhwa N (2010) Degradation of chicken feather a poultry waste product by keratinolytic bacteria isolated from dumping site at ghazipur poultry processing plant. *Int J Poult Sci* 9(5):482–489.

20. Bello, C. S. S., 2002. Laboratory manual for student of medical microbiology. *2nd ed. Jos: Satograhics Press*, pg. 80-85.

21. Odeyemi, A. T., Ayantola, K. J. and Peter, S. (2018). Molecular characterization of bacterial isolates and physicochemical assessment of well water samples from hostels at Osekita, Iworoko-Ekiti, Ekiti State. *American Journal of Microbiological Research*, *6*(1): 22-32

22. Osuntokun, O.T, Stephen, D.O, Akele EO, (2024). Emergence of pathogenic bacteria Isolates from zea maize extract using 16s rRNA molecular sequencing protocol as a tools for microbial identification and characterization. *J Bacteriol Mycol Open Access*;12(2):50 57. DOI: 10.15406/jbmoa.2024.12.00373

23. Uruku, M. N., Adikwu, I. A., Oyebola, O. O. and Akombo, P. M. (2021). Genetic diagnosis on strains of the African Catfish, Clarias gariepinus (Burchell 1822) in River Benue and a Tributary in North East Nigeria

24. Osuntokun, O,T, V. O. Azuh, O. A. Thonda and S. D. Olorundare (2024). Random Amplified Polymorphic DNA (RAPD) Markers Protocol of Bacterial Isolates from Two selected General Hospitals Wastewater (HWW). *Journal of Plant Biota*.3(1);28-33.

25. Osuntokun, O.T, Stephen, D.O, Akele EO, (2024). Emergence of pathogenic bacteria isolates from zea maize extract using 16s rRNA molecular sequencing protocol as a tools for microbial identification and characterization. *J Bacteriol Mycol Open Access*.12(2):50 57. DOI: 10.15406/jbmoa.2024.12.00373.

26. Osuntokun, O.T, Thonda, O A, Wilkie, D.(2004) Microbiological and Randomly Amplified Polymorphic DNA (RAPD) Marker Protocol for Silicone Condom Lubricant Isolates. *Sci .Tech Dev .J*.2024;27(2):3416-3430.

27. Mugoyela and Mwambete (2010) Mugoyela, V. and Mwambete, K.D. (2010) Microbial contamination of nonsterile pharmaceuticals in public hospital settings. Therapeutics and Clinical Risk Management, 6:443-448.

28. Ratajczak, M., Kubicka, M. M., Kamińska, D., Sawicka, P. and Długaszewska, J. (2015). Microbiological quality of non-sterile pharmaceutical products. *Saudi Pharmaceutical Journal*, *23*(3): 303-307.

29. Pal, M., Alemu, J., Mulu, S., Karanfil, O., Parmar, B. C. and Nayak, J. B. (2016). Microbial and hygienic aspects of dry milk powder. *Beverage and Food World*, *43*(7): 28-31.

30. Ben-David, A. and Davidson, C. E. (2014). Estimation method for serial dilution experiments. *Journal of microbiological methods*, *107*, 214-221.

31. Mukhtar, G. L. and Magashi, A. M. (2019). Assessment of microbial contamination in pediatric oral liquid formulations marketed in Katsina State, Nigeria. *Bayero Journal of Pure and Applied Sciences*, *12*(1): 657-662.

32. Huang, S. S., Datta, R. and Platt, R. (2006). Risk of acquiring antibiotic-resistant bacteria from prior room occupants. *Archives of internal medicine*, *166*(18), 1945-1951.

33. Bhusnure, O. G., Dongare, R. B., Gholve, S. B. and Giram, P. S. (2018). Chemical hazards and safety management in pharmaceutical industry. *Journal of Pharmacy Research*, *12*(3): 357-369

34. Jameel, F. (2017). Microbiological analysis of marketed available cough syrups in Karachi. *RADS Journal of Biological Research & Applied Sciences*, *8*(2): 05-10.

35. Byadgi, O. V., Rahmawaty, A., Wang, P. C. and Chen, S. C. (2023). Comparative genomics of *Edwardsiella anguillarum* and *Edwardsiella piscicida* isolated in Taiwan enables the identification of distinctive features and potential virulence factors using Oxford-Nanopore MinION® sequencing. *Journal of Fish Diseases*, *46*(4): 287-297.

36. Njoku, K. L., Asunmo, M. O., Ude, E. O., Adesuyi, A. A. and Oyelami, A. O. (2020). The molecular study of microbial and functional diversity of resistant microbes in heavy metal contaminated soil. *Environmental Technology and Innovation*, *17*, 100606.

37. Osuntokun, O.T., Ibukun, A.F., Yusuf-Babatunde, A.M. and Abiodun, S. (2019). Pre/post-plasmid profile analysis, killing- kinetics and secondary metabolites screening of *Adenopus brevifl orus* (Benth) fruit extract against Multiple Drug Resistant Isolates Using *Staphylococcus aureus* (MDRSA) as a case study. *J Adv Res Biotech*.4:1-17.