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Full Length Research Article

# MICROBIOLOGICAL QUALITY OF ANTI-TUBERCULOSIS DRUGS COMMONLY USED AT DOTS CENTRES AND PHARMACIES WITHIN KADUNA METROPOLIS, KADUNA, NIGERIA

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**ABSTRACT**
Medicinal drugs of low-quality likely due to microbial contamination can result in increased morbidity and mortality thereby reducing the human population and may also result in emergence of resistant strain organisms in the environment. Studies suggest that there are a lot of counterfeit and substandard anti-tuberculosis drugs in circulation especially in Africa that are used for the treatment and control of tuberculosis whose damage on public health were mostly underestimated. Research on microbiological quality of anti-TB drugs is regrettably inadequate and scarce with most of the researches focused only on determining the active pharmaceutical ingredients of the drugs. This research aimed at assessing the microbiological quality of the anti-TB drugs used at DOTS Centres and Pharmacies within Kaduna metropolis, specifically to determine the total aerobic microbial count, total combined yeast and mould counts, the presence of indicator organisms; *Escherichia coli, Salmonella species, Staphylococcus aureus and Pseudomonas aeruginosa* as well as the susceptibility of the isolates to standard antibiotics. A stratified random sampling was used. A total of fifty-two (52) samples comprising of Rifampicin, Pyrazinamide, Isoniazid, Ethambutol and 4FDC were collected for the study. Methods specified in the United States Pharmacopoeia (USP, 2019) under the test for specified organisms and enumeration test were adopted for this research work. The study conducted established that the anti-TB drugs used at DOTS centres and Pharmacies within Kaduna metropolis, Kaduna, Nigeria do not contain any of the indicator organisms but some of the selected anti-TB drugs are contaminated with other strains of microorganisms with some not meeting the requirement for total aerobic microbial count and total yeast and mould count. The microbial contaminants found include species of; *Bacillus, Klebsiella, Enterobacter, Penicillium, Fusarium, Alterneria, Curvularia, Aspergillus, Candida* and *Sporotricum*. Some of the selected isolates obtained from the selected anti-TB drugs were not susceptible to the standard antibiotics used; Augmentin, Chloramphenicol, Ciprofloxacin, Gentamicin, Amoxicillin and Ofloxacin, with highest resistance recorded in Amoxicillin for all the bacterial isolates. However, all the *Candida species* were susceptible to the antifungal drugs; Ketoconazole, Fluconazole, Clotrimazole and Itraconazole.

**Keywords:** Tuberculosis; Microbial count; DOTS; TB-drugs; 4FDC; Antibiotics.

**INTRODUCTION**
Tuberculosis is an airborne cosmopolitan disease that could be found in every human environment with its level of severity varying from one environment to another since 4000 BC (Adamu & McGill, 2018).

According to the world health organization, nearly 2 billion people, one third of the world population are infected with *Mycobacterium tuberculosis* with active cases of tuberculosis occurring in 7-8 million people annually with about 3 million death every year (Imam & Oyeyi, 2008). In 2019, the World Health Organization (WHO) estimated the number of tuberculosis infected people at 10 million of which 1.4 million died (WHO, 2020).

Susceptibility to tuberculosis is influenced by some environmental factors such as poor housing quality and overcrowding which are associated with poverty. Other factors include poor hygiene, poor sanitation, humidity, poor ventilation, high population of people living in the same environment, proximity and duration of the contact with people living with active-TB disease (Adamu & McGill, 2018; Kanchan *et al*., 2015).

Nigeria is among the top 20 countries with high burden of tuberculosis, high burden of TB-HIV co-infection and high burden of multiple drug resistant tuberculosis (MDR-TB) (Anonymous, 2017). Kaduna State is among the States with high prevalence rate in the North-West zone due to high level of human immunodeficiency virus (HIV) and combination of the high HIV prevalence and high poverty level are fuelling the TB endemic in the State (Gidado & Ejembi, 2009).

There is need for tuberculosis to be properly treated because of its negative impact on the environment because it primarily affects the most productive individuals and leads to reduction in human population and the economy (Kabir *et al*., 2010). Secondly the means through which tuberculosis is transmitted is a cause for concern as it is transmitted through inhalation of contaminated air that contain TB bacilli released to the environment either by sneezing, coughing, talking or spitting from infected individual (Anonymous, 2015). The National Tuberculosis Guidelines strongly recommended the use of Directly Observed Therapy (DOT) when treating persons with active tuberculosis. DOT implies that a trained and experienced health care worker or other designated individual provides the prescribed anti-TB drugs and watches the patient swallow every dose (Anonymous, 2017).

The emergence of resistance to drugs used in the treatment of tuberculosis and particularly multi drug-resistant tuberculosis (MDR-TB) is a significant problem and poses a great obstacle to effective tuberculosis control at both national and global levels (Anonymous, 2015). In 2016, there were 600,000 new cases of tuberculosis with resistance to Rifampicin (RR-TB), the most common effective first-line drug, of which 490,000 had multidrug resistance (MDR-TB) (i.e. resistance to at least two of the first-line drugs; Isoniazid, Ethambutol, Rifampicin and Pyrazinamide) (Anonymous, 2017).

Globally, there have been a serious concern about substandard antibiotics used for the treatment of tuberculosis in the developing countries, due to its significant effect on public health (Kelesidis & Falagas, 2015). It is reported that Nigeria is among the 28 different countries mostly in Asia and Africa were pharmaceutical products of low quality are used for the treatment and control of tuberculosis (Kelesidis & Falagas, 2015). Drugs sold in both open market and drug stores in Uyo metropolis Ibadan, showed contamination with pathogenic microorganisms including *S. aureus* and *Proteus mirabilis,* which constitute a health hazard to the public (Itah *et al.*, 2004). Similarly, high prevalence of poor-quality drugs was reported in Anambra, Nigeria by Onwujekwe *et al.* (2009).

Microbial contaminant, when present in pharmaceutical products, could interfere with the quality of the products by deactivating its potency (USP. 2019), and not only make them harmful from the infectious point of view, but may also alter the chemical, physical and organoleptic properties of the drugs or change the composition of the active ingredients. Furthermore, microorganisms can convert drugs to toxic products (Ratajczak *et al.*, 2014). Contaminated medicines may contain resistant non-pathogenic organisms that can spread resistance genes in the environment thereby posing health hazard to the population (Al-Charrakh, 2012). According to Kaniz *et al.* (2014), contamination of pharmaceutical drugs by microorganisms is as a result of some influencing factors which are but not limited to poor manufacturing practices, poor storage environment, contaminated raw materials, water used for manufacture, during transportation and distribution as a result of improper handling. Another important source of contamination to pharmaceutical drug is during unhygienic usage as well as the environment especially the air under which they are manufactured (Nigeria tuberculosis fact sheet, 2012; Kaniz, 2014).

Microbiological assessment of non-sterile pharmaceutical preparations is of utmost importance because microbial contamination can reduce or even eliminate the therapeutic effect of the medicine or cause drug induced infections (Ratajczak *et al.*, 2014). Non-sterile drugs must satisfy the acceptable microbiological criteria, which are included in the pharmacopoeial monograph. The presence of even a few numbers of disease-causing microorganisms, higher number of opportunistic pathogens or bacterial endotoxins which persist even after the death of the primary contaminants can render the product ineffective (Ratajczak *et al.*, 2014).

This research was aimed at determining the microbiological quality of anti-tuberculosis drugs commonly used at dots centres and pharmacies within Kaduna metropolis, Kaduna, Nigeria.

## MATERIALS AND METHODS

### Study Area

The study was conducted within Kaduna metropolis (comprising of Igabi, Kaduna North, Kaduna South and Chikun Local Government Areas). Kaduna has a population of 6,113,503 as at 2016 population projection with the population of Chikun, Igabi, Kaduna North and Kaduna South as: 502,500; 430,753; 492,100 and 543,600 respectively (Brinkhoff, 2017; Adamu & McGill, 2018).

### Ethical Approval

An Ethical approval with reference number MOH/ADM/744/VOL.1/615 was obtained from Kaduna State Ministry of Health before the commencement of the research work.

### Sampling Technique and Sample Size

A stratified random sampling was used. The study area was stratified into 4 strata (Chikun, Igabi, Kaduna North and Kaduna South Local Government Areas). A total of fifty-two (52) samples of anti-TB drugs, comprising of thirty-two (32) samples from four (4) DOTS centres within the selected locations and twenty (20) samples from two (2) Community Pharmacies were randomly collected.

### Sample Collection

Eight (8) samples comprising of; Pyrazinamide, Isoniazid, Ethambutol and 4-fixed-dose combination (two samples each) were collected in each of the DOTS centres. Ten (10) samples comprising of; Rifampicin, Pyrazinamide, Isoniazid, Ethambutol and 4-fixed-dose combination (two samples each) were collected in each of the two (2) Community Pharmacies. Each sample was put in a sterile polyethylene sample bag and was given a unique identification number. All samples were transported to National Agency for Food and Drug Administration and Control (NAFDAC) Kaduna Laboratory Services, Kaduna, where Laboratory analyses were conducted.

### Microbiological Assay of the Anti-TB Drugs

### Pre-testing procedure

Laboratory coat, hand gloves, Nose mask and eyeglasses were worn during the laboratory work. Material safety data sheet (MSDS) were consulted for the products used in order to be familiar with all precautionary measures before handling the samples. All materials used for microbial limit test were sterilized at 121 $^0$C for 15 minutes using an Osprey autoclave machine, where materials used are non-autoclavable, they were disinfected using 70 % ethanol. The temperature and humidity of the area were checked to ensure they are within limits of 30 $^0$C and 10% to 70 % respectively, before starting the activities. Environmental monitoring was carried out prior to and during analysis (United States Pharmacopoeia, 2019). All microbiological media used for the analyses were prepared in accordance with the manufacturer's instructions.

### Dilution of the Sample

The anti-TB drug samples were dissolved in Tryptic Soy Broth. About 1.0 g of each sample was weighed and was dissolved in 10.0 mL of sterile Tryptic Soy Broth (1 in 10 dilutions). Further dilutions, where necessary, were prepared using the same diluents (USP, 2019).

**Determination of total aerobic microbial count (TAMC)**
Determination of the total aerobic microbial count of the selected anti-TB drugs was done as described under the enumeration test, in the United States Pharmacopoeia (USP, 2019). About 9.0 mL each of Tryptic Soy Broth (TSB) was prepared into two McCartney bottles. It was sterilized and labelled with the dilution factor of $10^{-1}$ and the other labelled as diluent control. The prepared sample was homogenized in the prepared Tryptic Soy Broth. Sterile petridishes were labelled with the corresponding dilution factor of $10^{-1}$. Using a micropipette, 1.0 mL of the diluted sample was dispensed in duplicate in the sterile petridishes labelled TSA (Tryptic Soy Agar) also labelled with the identification number of the sample. About 20.0 mL of TSA was poured aseptically at about 45 °C into each of the petridishes. The inoculated sample and medium were mixed by swirling the plates gently. The Agar plates were allowed to solidify. All the plates were inverted and were aerobically incubated for 5 days at 32 ± 2 °C. After the completion of the incubation period, the plates were examined for the presence of colonies. Colonies on each of the plates were counted and the average count were multiplied by the dilution factor. Where the colonies on the plates are too numerous to count, the procedure was repeated but with higher dilution i.e. 1/1000, 1/10000 etc. and the average count was multiplied by the corresponding dilution factor. Where there was no colony at 1/10 dilution, results were reported as less than 10 CFU/g. The same procedure was used for each of the anti-TB drug samples (USP, 2019).

**Determination of total combined yeasts and moulds count (TYMC)**
The procedure described for TAMC was used for TYMC except that Sabouraud Dextrose Agar (SDA) was used instead of TSA and plates were incubated for 7 days at 22 ± 2 °C (USP, 2019). Colonies obtained on plates of Sabouraud Dextrose Agar were identified as yeast and mould by their morphological characteristics such as Pigmentation, Size of the colony, and pattern of their growth (Cheesbrough, 2009; Atlas of food microbiology lab, 2013).

**Control**
Appropriate controls such as; diluent, media and environmental controls were done as required under the enumeration test, in the USP (2019), as described below:

**Diluent control**
About 1.0 mL of the diluent (Tryptic soy broth) was plated out in duplicate on both Tryptic soy agar and Sabouraud dextrose agar plates. Plates of Tryptic soy agar were aerobically incubated for 5 days at 32 ± 2 °C. While, Sabouraud dextrose agar plates were incubated for 7 days at 22 ± 2 °C (USP, 2019).

**Media control**
Tryptic soy agar and Sabouraud dextrose agar used for the test were poured in sterile petridishes and were incubated alongside the test samples (USP, 2019).

**Environmental Control**
Two (2) empty sterile petridishes were labelled with TSA and SDA respectively and were left opened throughout the period of analysis. TSA and SDA media used for the test were poured into the Petridishes and were incubated alongside the test samples (USP, 2019).

**Isolation and Identification of *E. coli* and other indicator organisms in the selected anti-TB drugs**
Each of the anti-TB drug samples was examined for the presence of *E. coli, Salmonella spp, S. aureus and P. aeruginosa* as required under the test for specified organisms in the United States Pharmacopeia (USP, 2019).

**Isolation and Identification of *E. coli***
The anti-TB drug sample was prepared using a 1 in 10 dilution of not less than 1.0 g of the sample examined. About 10.0 g of the anti-TB drug was inoculated in 90.0 mL Tryptic soy broth (TSB). It was mixed and was incubated at 32 ± 2 °C for 24 h. The incubated sample was shaken after the incubation period, and 1.0 mL was transferred to 100.0 mL of MacConkey broth. It was incubated at 44 °C for 48 h. It was subcultured on a plate of MacConkey agar and was incubated at 32 ± 2 °C for 72 h. Growth of colonies indicates the possible presence of *E. coli.* A Pure colony was picked from the MacConkey agar plate and was inoculated on plate of Eosin Methylene Blue Agar (EMB). The plate was incubated for 72 h at 32 ± 2 °C. Where there was growth of colonies on EMB, it was confirmed by identification tests as described below under identification test. Where no growth of colonies is present or where the identification tests are negative, the product meets the requirement of the test (USP, 2019). The same procedure was used for each of the samples.

**Isolation and Identification of *Salmonella spp***
About 10.0 g of the anti-TB drug sample was inoculated in about 90.0 mL of Tryptic soy broth. It was mixed and incubated at 32 ± 2 °C for 24 h. Using a micropipette about 0.1 mL of the soya bean-casein digest broth previously inoculated with the sample was transferred to 10.0 mL of Rappaport Vassiliadis Salmonella enrichment broth and was incubated at 32 ± 2 °C for 24 h. It was subcultured on plates of Xylose lysine deoxycholate agar and was incubated at 32 ± 2 °C for 48 h. The possible presence of Salmonella is indicated by the growth of well-developed red colonies, with or without black centres. This was confirmed by identification tests. The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative (USP, 2019). The same procedure was used for each of the samples.

**Isolation and Identification of *S. aureus***
About 10.0 g of the anti-TB drug sample was inoculated in about 90.0 mL of Tryptic soy broth and was incubated at 32 ± 2 °C for 24 h. About 1.0 mL of the incubated sample was subcultured on a plate of Mannitol salt agar (MSA). It was incubated at 32 ± 2 °C for 72 hrs. The possible presence of *S. aureus* is indicated by the growth of yellow or white colonies surrounded by a yellow zone on MSA. This was confirmed by identification tests. The product complies with the test if such colonies are not present or if the confirmatory identification tests are negative (USP, 2019). The same procedure was used for each of the samples.

**Isolation and Identification of *P. aeruginosa***
About 10.0 g of the anti-TB drug sample was inoculated in about 90.0 mL of Tryptic soy broth. It was mixed and incubated at 32 ± 2 °C for 24 h. After the incubation period, 1.0 mL was subcultured on a plate of Cetrimide agar and was incubated at 32 ± 2 °C for 72 h. Growth of colonies indicates the possible presence of *P. aeruginosa.* This is confirmed by identification tests. (USP, 2019).

The same procedure was used for each of the samples.

**Acceptance criteria**

The acceptance criteria for test for the presence of *E. coli* is absence of the organism, the acceptance criteria for total combined yeasts/mould count (CFU/g) is $10^2$ while the acceptance criteria for total aerobic microbial count (CFU/g) is $10^3$ (USP, 2019).

**Identification of the other Bacterial isolates**

Other bacterial isolates on plates of Tryptic soy agar were identified. Twenty (20) plates with microbial growths were sorted out and were grouped based on their morphological characteristics. Subculture of some of the organisms from each group were done on plates of nutrient agar and Tryptic soy agar to obtain pure colonies of the isolates. The isolates were subcultured on some selective media and into nutrient broth to obtain a 24 h culture. Gram staining and Biochemical tests which include; Indole, Oxidase, Citrate utilization, Catalase, Coagulase, Methyl red, Mannitol motility test, Hydrogen sulphide production and Urease production were performed on each of the selected isolates following the method of Cheesbrough (2006).

**Identification of the Fungal Isolates on plates of Sabouraud dextrose agar**

Plates of Sabouraud dextrose agar with fungal growth were sorted out and 24 plates were randomly selected. Fungal contaminants of the anti-TB drugs were identified by their morphological characteristics which include; Pigmentation, Size of the colony, and pattern of their growth (Atlas of food microbiology lab, 2013), and were further identified by microscopy following the procedures described in Cheesbrough (2006).

**Determination of susceptibility of the bacterial isolates to some standard antibiotics**

This was done following the method of Kirby-Bauer disc diffusion method (Cheesbrough, 2006). Twenty-four (24) isolates previously identified as; *Bacillus, Enterobacter* and *Klebsiella species* were tested for their susceptibility to standard antibiotics. Mueller Hinton Agar was prepared and sterilized in accordance with the manufacturer's instruction. The medium was poured into 90.0 mm sterile Petridishes and was allowed to solidify before use. The antimicrobials included in the susceptibility testing were; Gentamycin, Augmentin, Ciprofloxacin, Ofloxacin, Amoxicillin, Chloramphenicol and Streptomycin. Susceptibility disc diffusion method was used. For each of the selected isolates, a sterile wire loop was used to pick 3 well-isolated colonies of the test organism and was emulsified in 9.0 mL of sterile nutrient broth. It was incubated for 24 h and its turbidity was visually adjusted to 0.5 McFarland standards. Two plates of Mueller Hinton agar were inoculated each with the 24 h broth using a sterile swab stick. The swabs were evenly streaked over the surfaces of the medium. The Petridishes were covered and were allowed to stay on the bench for the surface of the agar to dry. Using sterile forceps, the antibiotic discs were applied on the Mueller-Hinton Agar streaked with the inoculum. The plates were allowed to stay on the work bench for about 30 minutes before incubation. The plates were incubated aerobically at 32 ± 2 ℃ for 24 h. After the incubation period, the plates were examined for zones of inhibition. Using a ruler on the underside of the plates, the diameter of the zones of inhibition for each of the plates were measured in millimetre (mm) and the average was obtained. Using the Clinical Laboratory Standard Institute Interpretative Table, the zones sizes of each antimicrobial were reported as 'Susceptible', 'Intermediate' or 'Resistant' CLSI (2018).

**Antifungal susceptibility testing**

*Candida species* isolated from the anti-TB drugs were subjected to antifungal susceptibility testing. The test was done according to the methods of Mugoyela & Mwambete (2010) and NCCLS M44-A document, by disk diffusion method. Using a Whatman filter paper, 5 mm disc were punched and were impregnated with 25 µg of Fluconazole and 10 µg of; Clotrimazole, Itraconazole and Ketoconazole each, as these were not available in the form of antibiotic discs. About five (5) distinct colonies were picked from a 24-h old culture of *Candida species* from the Candida Selective agar plate. Colonies were then inoculated in 5 mL of sterile 0.85 % saline in a McCartney bottle, and its turbidity was visually adjusted to 0.5 McFarland standards. A sterile swab stick was used to pick an inoculum from the adjusted suspension within fifteen (15) minutes from the adjustment and was streaked on the surface of Mueller-Hinton agar (MHA) previously prepared according to the manufacturer's instruction. Excess moisture on the surface of the agar was allowed to be completely absorbed before adding the disks. Using a sterile forceps, Fluconazole disk (25 µg), Itraconazole (10 µg), Clotrimazole (10 µg) and Ketoconazole (10 µg) antifungal discs were applied on the Mueller-Hinton Agar, streaked with the inoculum and were incubated within 15 minutes of applying the disks. The plates were incubated in a VWR incubator, at 35 ± 2 °C for 24 h. The diameters of the zones of inhibition for each antifungal disk was measured in millimetres using a ruler, to the nearest whole millimetre at the point where there was a prominent reduction in growth. Interpretation of all antifungal susceptibility (Susceptible, Intermediate and Resistant) was done according to the CLSI standards.

**STATISTICAL ANALYSIS**

Data obtained from the research work were analysed using IBM SPSS version 25. Data for mean diameter of zones of inhibition obtained from the test for susceptibility of the bacterial isolates to some antibiotics, were analysed using a one-way ANOVA. For the percentage of samples outside the acceptable limit, chi-square was used to analyse the data.

**RESULTS**

The anti-TB drugs collected from the different DOTS centres and the community pharmacies within the selected locations shows contamination with microorganisms. The Average total aerobic microbial count and total yeast and mould count of the individual samples were presented in Table 1. The result showed that all the five categories of anti-TB drugs; Pyrazinamide, Ethambutol, Isoniazid, Rifampicin and 4-fixed-dose combination from the six (6) locations have contamination by both bacteria and fungi.

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**Table 1**: Total aerobic microbial count and total combined yeast and mould count (in CFU/g for the selected anti-TB drugs from the different locations

| Location | anti-TB drugs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pyrazinamide | | Ethambutol | | Isoniazid | | Rifampicin | | 4FDC | |
| | TAMC | TYMC | TAMC | TYMC | TAMC | TYMC | TAMC | TYMC | TAMC | TYMC |
| K/North | 15 | <10 | <10 | 20 | <10 | <10 | NA | NA | <10 | <10 |
| K/South | 20 | 50 | <10 | 50 | 50 | 5 | NA | NA | 150 | <10 |
| Chikun | <10 | 10 | 5 | 20 | <10 | <10 | NA | NA | 100 | 10 |
| Igabi | <10 | 15 | 2050 | 150 | 50 | 5 | NA | NA | 550 | 50 |
| Pharmacy X | 40 | 50 | 1550 | 20 | 2500 | 436 | 110 | 5 | 2900 | 160 |
| Pharmacy Y | 105 | <10 | 2275 | 105 | 60 | 5 | <10 | 110 | 115 | 5 |

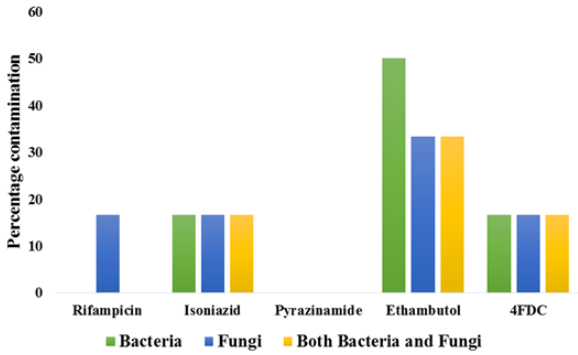
4FDC= 4 fixed-dose combination
CFU/g= Colony forming unit per gram
< = Less than
NA= Not Analysed
K= Kaduna

Table 2 shows the percentage of anti-tuberculosis drugs outside the acceptable limit for total aerobic microbial count (TAMC) and total combined yeast and mould count (TYMC), sampled from the six locations within Kaduna metropolis. The result indicates that percentage of samples above limit for total aerobic microbial count was highest among anti-tuberculosis drugs sampled from Pharmacy X (30.0 %) followed by those collected from Igabi LGA (12.5 %), then Pharmacy Y (10.0 %). However, the drugs collected from Kaduna North, Kaduna South and Chikun LGA all fall within acceptable limit for total aerobic microbial count. Similar trend was observed from the six locations for the total yeast and mould count, with the highest percentage of the drugs outside the acceptable limit of TYMC recorded in Pharmacy X (30 %), followed by that from Pharmacy Y (20 %), and Igabi LGA (12.5 %).

**Table 2**: Percentage of anti-tuberculosis drugs outside the acceptable limit for total aerobic microbial count and total combined yeast and mould count in the selected locations

| Location | Number examined | Percentage outside acceptable limit (%) | |
|---|---|---|---|
| | | Total aerobic microbial count | Total yeast and mould count |
| Kaduna North | 8 | 0.0 | 0.0 |
| Kaduna South | 8 | 0.0 | 0.0 |
| Chikun | 8 | 0.0 | 0.0 |
| Igabi | 8 | 12.5 | 12.5 |
| Pharmacy X | 10 | 30.0 | 30.0 |
| Pharmacy Y | 10 | 10.0 | 20.0 |
| *p* value | - | 0.031 | 0.001 |

The percentage of the individual samples contaminated with bacteria; fungi; both bacteria and fungi that falls above the acceptable limit were presented in Fig. 1. Highest contamination with bacteria was observed in Ethambutol (50.0 %), Isoniazid and 4FDC have 16.7 % each with the lowest in Rifampicin and Pyrazinamide (0.0 %) each. The result indicates that percentage above limit due to contamination with fungi was highest in Ethambutol (33.3 %), while Rifampicin, Isoniazid and 4FDC have 16.7 % each. The result also shows that 33.3 % of Ethambutol have both fungal and bacterial contamination that is outside the acceptable limit. The result also shows that 16.7 % of Isoniazid and 4FDC were outside the acceptable limit for both bacteria and fungi. Percentage above limit for Pyrazinamide was 0.0 %.




4FDC= 4 fixed-dose combination

**Fig. 1:** Percentage contamination for the selected anti-TB drugs

Fig. 2 shows the percentage of the anti-TB drugs selected in the DOTS Centres and the Community pharmacies that falls above the acceptable limit due to microbial contamination. The highest percentage above acceptable limit was recorded among samples from the Community pharmacies (20.0 %), and the lowest was recorded for the DOTS Centres (3.1 %).

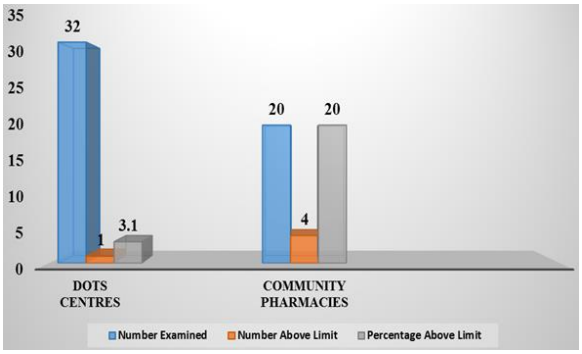



**Fig.2:** Percentage of the anti-TB drugs selected in the DOTS Centres and the Community Pharmacies, that falls above the acceptable limit.

The anti-TB drugs collected from the different DOTS centres and the Community pharmacies within the selected locations were not contaminated with any of the indicator organisms; *E. coli, S. aureus, P. aeruginosa,* and *Salmonella spp*. However, contamination with other microorganisms were found. The other microbial contaminants and their percentage occurrences were presented in Table 3. *Bacillus, Enterobacter* and *Klebsiella species* were found among the bacterial contaminants, while *Candida albicans, Aspergillus spp*, *Penicillium spp, Curvularia spp*, *Alterneria spp*, *Fusarium spp, Sporotricum spp* and *Saccharomyces spp* were found to be the fungal contaminants. The results showed that there was no statistically significant difference ($\chi^{2}_{(2)} = 2.250$; $p = 0.325$) in the occurrence of the three bacteria species identified as microbial contaminants of anti-TB drugs. *Bacillus* occurred in 45.8 % of all the sampled drugs, while *Enterobacter* and *Klebsiella* were identified in 33.3 and 20.8 % of

all the sampled drugs, respectively. However, there was a statistically significant difference ($\chi^2_{(4)} = 16.391$; $p = 0.003$) in the occurrence of fungal species contaminant in the anti-TB drugs. *Candida* was the most prevalent with 29.2 % occurrence, followed by *Curvularia*, *Alterneria* and *Fusarium* with an occurrence of 14.6 % each. *Aspergillus* and *Penicillium* were seen in 9.7 % and 7.3 % of all samples, respectively while *Sporotricum* and *Saccharomyces* were present in 4.9 % of all sampled drugs.

**Table 3:** Other microbial contaminants in the selected anti-TB drugs and their percentage occurrence

| Bacterial species | | Fungal species | |
|---|---|---|---|
| Organism | % occurrence | Organism | % occurrence |
| Bacillus | 11 (45.8) | Aspergillus | 4 (9.7) |
| Enterobacter | 8 (33.3) | Candida | 12 (29.2) |
| Klebsiella | 5 (20.8) | Penicillium | 3 (7.3) |
| - | - | Curvularia | 6 (14.6) |
| - | - | Alterneria | 6 (14.6) |
| - | - | Fusarium | 6 (14.6) |
| - | - | Sporotricum | 2 (4.9) |
| | | Saccharomyces | 2 (4.9) |

% = Percentage; Bacteria ($\chi^2_{(2)} = 2.250$; $p = 0.325$); Fungi ($\chi^2_{(4)} = 16.391$; $p = 0.003$)

Results of the susceptibility testing done on some of the bacterial isolates against some antibiotics were shown in Table 4. Findings from the results showed that there was a statistically significant difference ($\chi^2_{(12)} = 29.873$; $p = 0.003$) in the susceptibility pattern of *Bacillus species* to the antibiotic drugs and were found to be most susceptible to Ofloxacin (100%) followed by Streptomycin and Ciprofloxacin (90.9 %, each), then followed by Augmentin and Gentamycin (81.8 %, each). About 72.7 % of the isolated *Bacillus species* were susceptible to Chloramphenicol while least susceptibility was seen with Amoxicillin (18.2 %). The isolated *Bacillus species* were resistant to three drugs; Amoxicillin (18.2 %), Augmentin (9.1 %) and Gentamycin (9.1 %). Similarly, significant difference ($\chi^2_{(12)} = 25.375$; $p = 0.013$) in antibiotic susceptibility pattern of the isolated *Enterobacter species* was observed. *Enterobacter species* were 100 % susceptible to the effects of Chloramphenicol, Ciprofloxacin, Gentamycin, and Ofloxacin, with least susceptibility seen with Amoxicillin. Resistance of *Enterobacter species* was observed with Amoxicillin (25 %). Furthermore, there was a statistically significant difference ($\chi^2_{(12)} = 91.497$; $p < 0.001$) in the susceptibility pattern of *Klebsiella species* to the antibiotic drugs that were tested. The isolated *Klebsiella species*, were most susceptible to the actions of Augmentin, Ciprofloxacin, and Ofloxacin (100 %, each) followed by Streptomycin and Gentamycin (80 %, each), then followed by Chloramphenicol (60 %, each). The least susceptibility was seen with Amoxicillin (20 %). The isolated *Klebsiella species* were resistant to one drug; Amoxicillin (40 %).

**Table 4:** Susceptibility of the isolated bacterial contaminants to some standard antibiotics

| | Bacterial specie tested | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Bacillus | | | Enterobacter | | | Klebsiella | | |
| Antibiotic Disc (Strength) | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) |
| Amoxicillin (10 µg) | 2 (18.2) | 7 (63.6) | 2 (18.2) | 3 (37.5) | 3 (37.5) | 2 (25) | 1 (20) | 2 (40) | 2 (40) |
| Augmentin (30 µg) | 9 (81.8) | 1 (9.1) | 1 (9.1) | 7 (87.5) | 1 (12.5) | 0 (0) | 100 (100) | 0 (0) | 0 (0) |
| Chloramphenicol (30 µg) | 8 (72.7) | 3 (27.3) | 0 (0) | 8 (100) | 0 (0) | 0 (0) | 3 (60) | 2 (40) | 0 (0) |
| Ciprofloxacin (5 µg) | 10 (90.9) | 1 (9.1) | 0 (0) | 8 (100) | 0 (0) | 0 (0) | 5 (100) | 0 (0) | 0 (0) |
| Gentamicin (10 µg) | 9 (81.8) | 1 (9.1) | 1 (9.1) | 8 (100) | 0 (0) | 0 (0) | 4 (80) | 1 (20) | 0 (0) |
| Ofloxacin (5 µg) | 11 (100) | 0 (0) | 0 (0) | 8 (100) | 0 (0) | 0 (0) | 5 (100) | 0 (0) | 0 (0) |
| Streptomycin (10 µg) | 10 (90.9) | 1 (9.1) | 0 (0) | 6 (75) | 2 (25) | 0 (0) | 4 (80) | 1 (20) | 0 (0) |

S = Susceptible, I = Intermediate, R = Resistant, % = Percentage

Table 5. Shows the mean diameter of zone of inhibition for the isolated organisms. The result showed that there was statistically significant difference in the zones of inhibition of the different antibiotics used against the bacterial isolates

**Table 5:** Mean diameter of zone of inhibition for the isolated organisms (mm)

| | Antibiotic used | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Organism tested | Ciprofloxacin (5 µg) | Augmentin (30 µg) | Gentamicin (10 µg) | Ofloxacin (5 µg) | Amoxicillin (10 µg) | Chloramphenicol (30 µg) | Streptomycin (10 µg) | *P*-value |
| *Bacillus* sp | 29.2±4.64$^{d}$ | 18.6±2.54$^{ab}$ | 20.6±6.21$^{b}$ | 25.6±3.61$^{c}$ | 15.7±1.90$^{a}$ | 19.6±3.29$^{b}$ | 18.4±1.91$^{ab}$ | <0.001 |
| Enterobacter sp | 32.4±2.33$^{d}$ | 19.8±1.49$^{b}$ | 19.9±3.68$^{b}$ | 24.3±5.15$^{c}$ | 15.4±2.62$^{a}$ | 20.3±1.91$^{b}$ | 17.5±2.67$^{ab}$ | <0.001 |
| Klebsiella sp | 30.8±1.48$^{d}$ | 21.2±2.78$^{c}$ | 17.6±2.88$^{ab}$ | 25.2±4.32$^{c}$ | 14.6±2.61$^{a}$ | 18.0±2.92$^{ab}$ | 16.8±2.39$^{a}$ | <0.001 |

Values are given as mean ± standard deviation of mean. In each row, mean values with different superscripts have statistically significant difference ($p < 0.05$)

*P*-Value = Probability value
mm = Millimetre
µg = Microgram

The result of the antifungal susceptibility testing done on *Candida species* was presented in Table 6. The results showed that there was no statistically significant difference ($\chi^2_{(3)} = 3.064$; $p = 0.382$) in the susceptibility pattern of the Candida isolates to the four antifungal drugs tested. None of the isolate was resistant to the drugs. One isolate had intermediate susceptibility to Clotrimazole. All twelve isolates were susceptible to the antifungal effects of Fluconazole, Ketoconazole and Itraconazole, while 11 out of 12 isolates were susceptible to Clotrimazole.

**Table 6:** Antifungal susceptibility pattern of Candida isolates obtained from the anti-TB drugs tested

| Antifungal drug used | Susceptible (%) | Intermediate (%) | Resistant (%) |
|---|---|---|---|
| Fluconazole | 12 (100) | 0 (0) | 0 (0) |
| Clotrimazole | 11 (91.6) | 1 (8.3) | 0 (0) |
| Ketoconazole | 12 (100) | 0 (0) | 0 (0) |
| Itraconazole | 12 (100) | 0 (0) | 0 (0) |

% = Percentage; ($\chi^2_{(3)} = 3.064$; $p = 0.382$)

Plates. 1, 2 and 3 shows the zones of inhibition by some of the antibiotics used against *Bacillus, Enterobacter* and *Klebsiella species* respectively on plates of Mueller Hinton agar. The antibiotics used against the bacterial isolates include; Augmentin, Amoxicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Streptomycin and Ofloxacin. Plate. 4 shows the zones of inhibition by some of the antifungal drugs used against *Candida species* on plates of Sabouraud dextrose agar. The antifungal drugs used were; Fluconazole, Clotrimazole, Ketoconazole and Itraconazole.

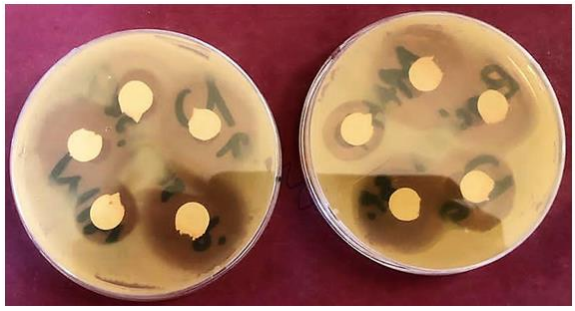


**Plate 1:** Zones of inhibition of some of the antibiotics used against *Bacillus specie* on plates of Mueller Hinton agar

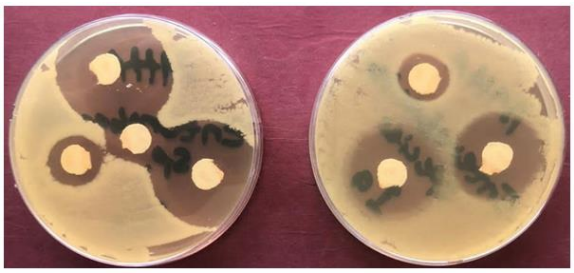


**Plate 2:** Zones of inhibition of some of the antibiotics used against *Enterobacter specie* on plate of Mueller Hinton agar

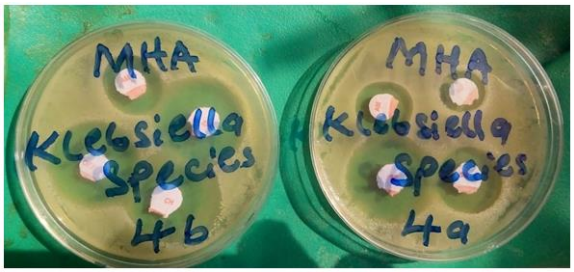



**Plate 3:** Zones of inhibition of some of the antibiotics used against *Klebsiella specie* on plates of Mueller Hinton agar


**Plate 4:** Zone of inhibition by some of the antifungal drugs used against *Candida species* on plates of Sabouraud dextrose agar

**DISCUSSION**

This research work has established the presence of microorganisms in the selected anti-TB drugs commonly used for the treatment of tuberculosis at DOTS centres and Community Pharmacies within Kaduna metropolis, Kaduna, Nigeria, with some exceeding the acceptable pharmacopoeial limits of $10^3$ CFU/g and $10^2$ CFU/g for both total aerobic microbial count and total combined yeast and mould counts respectively. The most common bacterial contaminants found include; *Bacillus species, Klebsiella species and Enterobacter species*. While, *Penicillium species, Fusarium species, Alterneria species, Curvularia species, Aspergillus species, Candida albicans, Saccharomyces species* and

*Sporotricum species* were the most common fungal contaminants found in the anti-TB drugs tested. The presence of microbial contamination in the drugs could indicate poor hygienic practices either during manufacture, packaging or handling by end-users which potentially exposes them to postproduction contamination by microorganisms. The drugs could also get contaminated by microorganisms in the environment. Findings from this research work agrees with that of Obuekwe *et al.* (2001), where they reported both fungal and bacterial contamination in non-prescription tablet drugs in Nigeria and Kuwait. Similarly, Adeola *et al.* (2012), reported that tablets sampled from Community Pharmacies within Lagos, Nigeria, were microbiologically contaminated with both bacteria and fungi such as *Micrococcus species*, *Staphylococcus aureus*, *Clostridium species*, *Aspergillus species*, *Penicillium species* and *Microsporum species*. In a similar study by Aghili *et al.* (2016), conducted on some tablets and ointments used in Hospitals showed that 70.3 % of the tablets have microbial contamination, with *Aspergillus species* as the most common fungal contaminants. All the samples collected from the DOTS centres conform to the requirement for the enumeration test except one of the samples in one of the locations (Igabi) which falls above the acceptable limit of $10^3$ and $10^2$ CFU/g for total aerobic microbial count and total combined yeast and mould counts respectively, as specified in the United States Pharmacopeia. In the community pharmacies samples from one of the pharmacies (pharmacy X) have the higher number of drugs that fall outside the acceptable limit of $10^3$ and $10^2$ CFU/g for both bacterial and fungal count followed by samples from pharmacy Y. For the individual samples, Rifampicin have the highest contamination followed by Isoniazid, Ethambutol and 4FDC, while all the Pyrazinamide samples fall within the acceptable limit. The research work based on this finding has established the presence of poor-quality anti-TB drugs in the selected locations. The percentage of the samples above the acceptable limit is low for the DOTS centres compared to that of the community pharmacies but for anti-TB drugs to contain microorganisms it is a cause for concern because they could be an avenue through which resistant genes and resistant strain organisms are spread in the environment thereby promoting global microbial resistance which in turn tender antimicrobials used for the treatment of diseases ineffective as reported by Mukhtar *et al*. (2020).

The high percentage of samples above the acceptable limit, recorded for the community pharmacies may be attributed to the nature of the drug's packaging materials and/or through handling as most of the drugs collected were loose tablets in a plastic container which are counted at the point of sale and this may lead to contamination with microorganisms in the environment. Most of the tablets are normally placed on a tray and returned to the container when the desired number is taken. However, Obuekwe *et al.* (2001), reported microbial contamination on the surfaces of tablets irrespective of the packaging material or coating. The microbial contamination recorded in this research work could also be due to postproduction contamination from both handlers and the environment. In the work of Obuekwe *et al* (2001) scanning electron microscopy showed that 64 % of tablet samples from Nigeria and Kuwait have superficial contamination by microorganisms. Similarly, in the work of Aghili *et al.* (2016), microbial contamination in pharmaceutical drugs that were properly packaged was reported. This drugs most have gotten contaminated by the packaging material, raw material used for production, or unhygienic environment under which they were produced.

Quality of pharmaceutical drugs is determined by number of factors which includes good manufacturing practices, use of good raw materials, good packaging materials, proper storage condition, good handling and distribution processes after manufacture of the product. Failure to adhere to any of the above or other good practices could result to producing pharmaceutical drugs of poor quality which facilitates treatment failure and drug resistance.
The results of the determination of *E. coli* and other indicator organisms in the anti-TB drugs examined shows that none of the samples contained *E. coli* or any of the indicator organisms; *Salmonella, S. aureus, P. aeruginosa.* This finding is in agreement with that of Atata & Biyaosi (2016), where some tablet and syrup drugs from Nigeria showed contamination with microorganisms including *Bacillus* and *Candida species* but none of the indicator organisms; *E. coli, Salmonella spp, S. aureus* and *P. aeruginosa* were isolated. Similarly, Shaqra *et al.* (2014), have similar findings in their work on blister-packed tablets manufactured in Jordan in which they reported that specified objectionable bacteria such as *E. coli* and *S. aureus* were not detected. They also isolated *Aspergillus species* and *Penicillium species* in small numbers and found Bacillus as the most predominant species. One of the quality indicators of a medicinal drug especially those administered orally, is the absence of *E. coli, Salmonella, S. aureus* and *P. aeruginosa* as stated under test for specified organisms (USP, 2019).

The results of the susceptibility testing showed that some of the selected isolates obtained from the selected anti-TB drugs were not susceptible to the antibiotics used with all the three species; *Bacillus, Enterobacter* and *Klebsiella* showing resistance to Amoxicillin. However, majority of the isolates were found to be susceptible to the drugs; Amoxicillin, Augmentin, Chloramphenicol, Ciprofloxacin, Gentamycin, Ofloxacin, and Streptomycin. Findings from this research work agrees with that of Essam *et al.* (2013), where they reported that 100 % of isolates from drugs were susceptible to Ciprofloxacin and about 21.4 % were not susceptible to Amoxicillin. Findings from this research work is also in agreement with that of Kaniz *et al*. (2014) where they reported antibiotic resistance in some bacterial isolates obtained from oral drug samples. However, this finding disagrees with that of Mukhtar *et al*. (2020), where they reported high-level of resistance to Amoxicillin/Clavulanic (Augmentin) by bacterial strains subjected to them. Similarly, the finding also disagrees with that of Mugoyela & Mwambete (2010) where some isolates from pharmaceutical drugs showed resistance to Augmentin and Cloxacillin. The presence of resistant strain organisms in the selected anti-TB drugs is a potential problem in the environment and poses a threat to effective tuberculosis treatment (Mukhtar *et al*., 2020).

The results of the antifungal susceptibility testing done on *Candida species* showed an overall susceptibility to all the antifungal drugs used; Fluconazole Ketoconazole and Itraconazole. However, one isolate showed an intermediate susceptibility to Clotrimazole with none of the isolates showing resistance to any of the drugs. This finding is in agreement with that of Mugoyela & Mwambete (2010), where *Candida species* subjected to Fluconazole and Ketoconazole antifungal drugs were found to be susceptible to both drugs. However, the findings disagree with the findings of Giri & Kindo (2014) where they reported 30.8 % and 12.8 % resistance

by Candida isolates to Fluconazole and Ketoconazole respectively. Although all the *Candida species* isolated from the anti-TB drugs were found to be susceptible to all the antifungal drugs used, there is still a need to adhere to good manufacturing and distribution practices because of they are opportunistic pathogens of humans and their presence in drugs is not desirable as stated in the United State Pharmacopeia.

Proper storage conditions, good hygiene and distribution practices should always be adhered to at both DOTS Centres, Pharmacies and Medical stores. The drug regulatory agency in the country (NAFDAC) and other relevant agencies should be adequately provided with all the necessary legislations and funding to carryout post marketing surveillance on all anti-TB drugs and other pharmaceutical products locally produced or imported. Interventions against tuberculosis should include regular quality assessment of anti-TB drugs used for the treatment of tuberculosis by providing researchers in research as well as learning institutes with adequate funding. Further studies that will determine the presence of β-lactamase in isolates from anti-TB drugs and profiling of plasmids which is important in transmission of the β-lactamase gene among bacterial strains should be conducted in order to have a better understanding of antimicrobial resistance and its spread in the environment.

**Conclusion**

The study conducted established that some of the selected anti-TB drugs used at DOTS centres and Community Pharmacies within Kaduna metropolis, Kaduna, Nigeria are contaminated with microorganisms, with some not meeting the USP (2019) requirements, for total aerobic microbial count and total yeast and mould counts. The study also established the absence of *E. coli. S. aureus, Salmonella* and *P. aeruginosa* in the anti-TB drugs examined meanwhile *Candida species* were isolated. Some of the selected bacterial isolates obtained from the selected anti-TB drugs were not susceptible to the standard antibiotics used. However, all the *Candida* isolates were susceptible to all the antifungal drugs. The study has shown that a reasonable number of the anti-TB drugs (Isoniazid, Pyrazinamide, Ethambutol and 4FDC) from the selected DOTS centres within Kaduna metropolis during the period of the study are of good quality. However, several anti-TB drugs from the Community Pharmacies in the study area at the period of the research are of poor quality.

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**REFERENCES**

Adamu, S.U. & McGill, D. (2018). Drugs Supply and Laboratory Services in Dots System of Kaduna State: A Health Care Perspective. *Journal of Tuberculosis Research,* 6, 19-35. https://doi.org/10.4236/jtr.2018.61003

Adeola, A.R., Opara, M.I. & Adeleye, I.A. (2012). Microbial quality of some non-sterile pharmaceutical products sourced from some retail pharmacies in Lagos, Nigeria. African Journal of Microbiology Research. **6**(23): 4903-4907. http://www.academicjournals.org/AJMR DOI: 10.5897/AJMR11.1158

Aghili S. R., Hossein nejad, A., Jabbari Amiri, M. R. & Abastabar, M. (2016). Risk of Fungal Contamination of Ointments and Tablets after Opening of the Package for Use in Hospitals. *Iranian Journal of Health Sciences. **4**(4):*1-13. Doi: 10.18869/acadpub.jhs.4.4.1.

Al-Charrakh, A. H. (2012). Frequency and antimicrobial resistance of bacteria isolated from oral and topical medicaments from Hilla, Iraq. *The Journal of Infection in Developing Countries*, *6*(06): 489–494. https://doi.org/10.3855/jidc.1817

Anonymous (2017*).* Directly observed therapy (DOT) for the treatment of tuberculosis. Minnesota Department of Health. Retrieved December 28, 2017 from www.healthstate.mn.us/tb

Anonymous (2015). *National Tuberculosis, Leprosy and Buruli ulcer Management and Control Guidelines*. 6th Edition.

Atata, R.F. & Biyaosi, S.Y. (2016). Microbiological Quality of Some Selected Brands of Tablets and Syrups Produced in Nigeria. *The Pharmaceutical and Chemical Journal*. ***3***(3):17-20.

Atlas of Food Microbiology LAB Microorganisms including: Bacteria, Molds & Yeast (2012). University of Baghdad-College of Science-Department of Biology Forth Classes 2012- 2013 1st. Electronic Edition–for Limited use only.

Brinkhoff T. (2017). City population- statistics, maps and charts. Retrieved from https://citypopulation.de/php/nigeria-admin.php?adm1id=NGA019.

Cheesbrough M. (2006). *District Laboratory Practice in Tropical Countries part 2*. Cambridge University Press. Cambridge Low Price Edition Pp. 132-141.

Clinical and Laboratory Standards Institute (2018). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 10th ed. M07-A11. Clinical and Laboratory Standards Institute, Wayne, PA.

El-Housseiny, R.S., Aboulwafa, M.M., El-Khatib, W.F. & Hassouna, N.A.-H. (2013). Recovery and detection of microbial contaminants in some non-sterile pharmaceutical products. *Archives of clinical microbiology. **4***(6): 1-14. Doi: 10.823/278.

Essam A. M., Mohamed M. I. & Mamdoh S. E. (2013). Presence of Antibiotic Resistant Bacteria along the Pharmaceuticals Production Line. Malaysian Technical Universities Conference on Engineering and Technology 2012, MUCET 2012 Part 6 - Science *Procedia Engineering 53: 715 – 721* doi: 10.1016/j.proeng.2013.02.094

Gidado, M. & Ejembi, C.L. (2009). Tuberculosis case management and treatment outcome: Assessment of the effectiveness of public-private mix of tuberculosis programme in Kaduna State, Nigeria. (Vol 8). http://doi:10.4103/1596-519.55760

Giri, S. & Kindo, A.J. (2014). Evaluation of antifungal susceptibility testing in Candida isolates by Candifast and disk-diffusion method. *Indian Journal of Pathology and Microbiology.* ***57***(4):595-7. *http://www.ijpmonline.org*. doi: 10.4103/0377- 4929.142680.

Imam, T. S., & Oyeyi, T. I. (2008). A retrospective study of Pulmonary Tuberculosis (PTB) prevalence amongst patients attending infectious diseases hospital, in Kano, Nigeria. *Bayero Journal of Pure and Applied*

*Sciences*, ***1***(1), 10–15.

Itah, A.Y., Udokpoh, A. E., & Ofum, M. U. (2004). Bacteriological quality of some pharmaceutical products marketed by drug vendors in Uyo, Nigeria. *African Journal of Health Sciences*, ***11***(3), 128–133. https://doi.org/10.4314/ajhs.v11i3.30789

Kabir M., Obasanya O. & Van der Grinten E. (ed.) (2010*). National Tuberculosis and Leprosy Control Programme. Worker's Manual Revised 5th Edition.*

Kanchan S., Surya K., & Ajay V. (2015). Role of Environmental factors in Transmission of Tuberculosis. *Journal of Health 2*(4). Retrieved from http://journalofhealth.co.nz/? Page id=982.

Kaniz F., Sowmitra R.C., Tohora S, Mokhlesur R.M.D., Niaz M.K., Kamal K.D. & Rashed N. (2014). Assessment of microbiological quality of the pediatric oral liquid drugs *Journal of Pharmacognosy and Phytochemistry 2014; **3**(1): 165-171.*

Kelesidis, T., & Falagas, M. E. (2015). Substandard/Counterfeit Antimicrobial Drugs. *Clinical Microbiology Reviews*, ***28***(2), 443–464. https://doi.org/10.1128/CMR.00072-14

Mugoyela, Veronica, & Kennedy D Mwambete. "Microbial Contamination of Nonsterile Pharmaceuticals in Public Hospital Settings." *Therapeutics and Clinical Risk Management* ***6***(2010): 443–48. https://doi.org/10.2147/TCRM.S12253.

Mukhtar G.L., Mukhtar M.D. & Magashi A.M. (2020). Antimicrobial resistance among bacterial strains recovered from non-sterile pharmaceutical products. *African Journal of Biology and Medical Research*. 3(3): 1-11.

Nigeria tuberculosis factsheet (2012). by United States embassy in Nigeria. http://nigeria.usembassy.gov

NCCLS (2004). Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline. NCCLS document M44-A [ISBN 1-56238-532-1]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

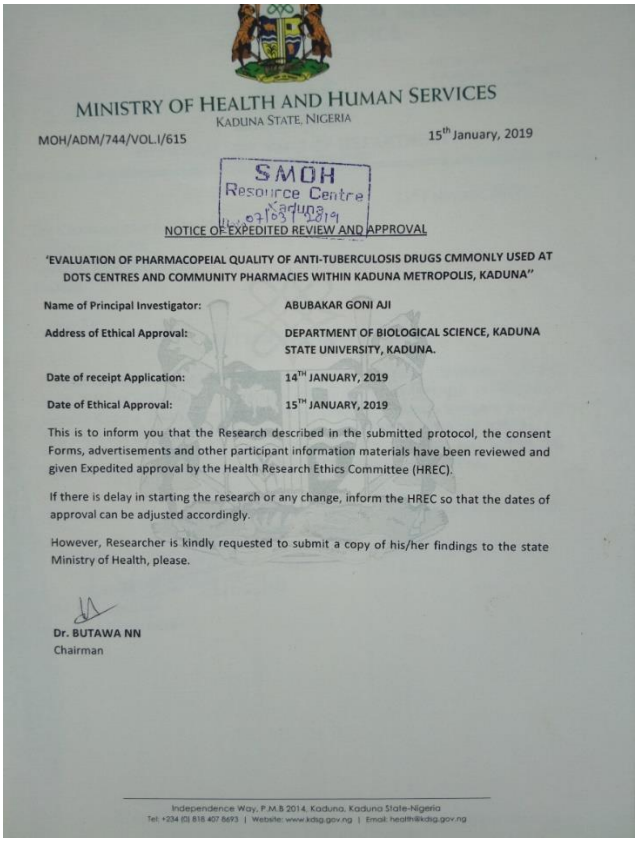
Obuekwe C.O., Obuekwe I.F. & Rafiq M. (2001). Surface microbial contamination in some commonly available tablet dosage forms. Medical Principles and Practice. 2000; 9:290-299.

Onwujekwe, O., Kaur, H., Dike, N., Shu, E., Uzochukwu, B., Hanson, K., & Okonkwo, P. (2009). Quality of anti-malarial drugs provided by public and private healthcare providers in south-east Nigeria. *Malaria Journal*, *8*, 22. https://doi.org/10.1186/1475-2875-8-22

Ratajczak M., Kubicka M.M, Kamin´ska D., Sawicka P., & Długaszewska J. (2014). Microbial quality of non-sterile pharmaceutical products. *Saudi Pharmaceutical Journal* 23, 303-307. Retrieved from www.sciencedirect.com

Shaqra, Q.M.A., Shawaqfah, M.T. & Al Momani, W. (2014). Microbiological Quality of Blister Pack Tablets in Community Pharmacies in Jordan. *Tropical Journal of Pharmaceutical Research*. ***13***(2): 261-266. http://www.tjpr.org

U.S Pharmacopeia (2019). *National formulary. USP 40 NF 35* Vol. 1. The United State Pharmacopeial Convention 12601 Twin brook BRIParkway, Rockville, MD 20852. Pp. 771



MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.I/615 — 15th January, 2019

SMOH Resource Centre Kaduna

NOTICE OF EXPEDITED REVIEW AND APPROVAL

'EVALUATION OF PHARMACOPEIAL QUALITY OF ANTI-TUBERCULOSIS DRUGS CMMONLY USED AT DOTS CENTRES AND COMMUNITY PHARMACIES WITHIN KADUNA METROPOLIS, KADUNA"

| | |
|---|---|
| Name of Principal Investigator: | ABUBAKAR GONI AJI |
| Address of Ethical Approval: | DEPARTMENT OF BIOLOGICAL SCIENCE, KADUNA STATE UNIVERSITY, KADUNA. |
| Date of receipt Application: | 14TH JANUARY, 2019 |
| Date of Ethical Approval: | 15TH JANUARY, 2019 |

This is to inform you that the Research described in the submitted protocol, the consent Forms, advertisements and other participant information materials have been reviewed and given Expedited approval by the Health Research Ethics Committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the state Ministry of Health, please.

Dr. BUTAWA NN
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