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Bacteriological Screening of Paediatric Cough Syrups Marketed Within Port Harcourt Metropolis, South-South Nigeria

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ABSTRACT

Non-adherence to good manufacturing practice alongside improper handling during dispensing, packaging and inadequate post-marketing surveillance of pharmaceutical products accounts to product's deterioration reduced therapeutic effect and adversely affects patient's safety especially the paediatrics. This study evaluates the microbiological quality of various brands of paediatric cough syrups marketed and used within Port Harcourt metropolis. Twenty cough syrup brands were experimented on in duplicate, coded as USS and UNS (used and unused respectively). They were subjected to organoleptic assessment, pH, viscosity, total aerobic viable count, as well as resistance- susceptibility test of isolates using standard conventional techniques. Results showed viscosity value of 0.22 - 9.09 Pascal seconds (Pa.s), pH values of 3.13 - 8.34 across the UNS and USS categories respectively. While 80% of the UNS cough syrup samples were free from potential microbial threat, 20% fraction and all USS cough syrup (100%) samples were contaminated with objectionable microorganisms and non-compliant with USP permissible limit. The potentially pathogenic isolates were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia,* demonstrating varied resistance pattern to exposed antibiotic categories. Microbial contamination might have been caused by poor quality control and improper handling of the products during use. This calls for more stringent measures during product manufacturing and handling to ensure patient's safety and forestall possible transference of resistance strain.

Keywords: Pathogenic, Paediatric, Cough syrup, Contamination, Resistance.

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INTRODUCTION

The use of pleasanty coloured, flavoured and appropriately sweetened liquid dosage form has been a mainstay practice in the treatment of disease conditions in paediatric and geriatric age groups. These linctuses (cough syrups) are viscous, non-sterile pharmaceutical

preparations commonly prescribed for the relief cough. Coughing is the rapid expulsion of air typically from the lungs in order to clear it of fluid, irritants, foreign particles and microbes. [1] In children, it could be productive or unproductive and often the most common reasons for which parents seek medical attention for their children. [2] Since many additives in these preparations are ready substrates for microbial growth [3], their manufacture, storage and distribution must conform to Good Manufacturing Practice (GMP). [4-5] As a non-sterile formulation, their microbial load must be within the acceptable pharmacopoeial limit and free from objectionable microorganisms; [6] *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella species, Klebsiella pneumoniae, Candida albicans*. [4-7] Additionally, the syrup should be capable of maintaining low contamination levels during use [8] since presence of these organisms can reduce or even affect the product's quality, therapeutic activity and constitute a potential danger to patient's health. [9-10]

A study on determination of microbial load in multivitamin and cough syrups sold in Dhaka City by Al-Mamun *et al*., indicated that out of 75% contaminated cough syrups tested, the major contaminants were *Staphylococcus aureus* (75%), *Escherichia coli* (17%) and Total coliform (42%). [11] Similar study on the microbial contamination of non-sterile pharmaceuticals in public hospital settings by Mugoyela & Mwambete showed that 50% of all tested products were heavily contaminated with *Klebsiella*, *Bacillus*, and *Candida* as the predominant species [4] Their susceptibility experiment confirmed the resistant of these isolates to Amoxicillin/clavulanic acid and Cloxacillin. Large bioburden of microbial contamination associated with cough syrups has been attributed to low concentration of sucrose in the finished product, non compliance with Good Manufacturing Practice and quality of the raw material. [12]

In recent times, there has been concerned about pharmaceutical products including oral paediatric drugs being contaminated by undesirable organisms within a short period of their usage. [11] Previous reports have highlighted the possibility that these undesirable organisms would during their shelf life contribute to physical deterioration of the product, reduce the therapeutic activity of the product which could affect the health of the patients. [11] Again, from a public health point of view, there is also the need for continuous studies to identify objectionable microorganisms present in non-sterile products as a way of ascertaining its safety. This study therefore evaluates the microbiological and physico-chemical qualities of various brands of paediatric cough syrups within Port Harcourt, to determine presence of objectionable microorganisms and their susceptibility to commonly prescribed antibiotics.

MATERIALS AND METHODS

Culture Media and antibiotics

The following media were used as procured and include: Nutrient broth, Peptone water, Nutrient agar, Cetrimide agar, Mac-Conkey agar, Mannitol salt agar (Titan Biotech, India). The media were reconstituted according to manufacturer's instructions and ready for use. [13] The Antibiotic discs used were as follows: ceftriaxone 30µg, cloxacillin 5µg, ofloxacin 5µg, erythromycin 5µg, gentamicin 10µg, cefuroxime 30µg, ceftazidime 30µg, amoxicillin/clavulanic acid 20/10µg, nitrofurantoin 300µg, ampicillin10µg and ciprofloxacin 5µg (Roche, Germany).

Sample collection

Duplicates of twenty (20) brands of cough syrups frequently prescribed for children were randomly purchased from approved pharmacy outlets within Port Harcourt Metroplolis in South-south Nigeria. They were transported to pharmaceutical microbiology laboratory and evaluated within 24 hours. They were physically examined for manufacturing and expiry dates as well as National Agency for Food and Drug Administration and Control (NAFDAC) Registration Numbers.

Evaluation Protocol

A total of forty (40) samples comprising twenty (20) each of UNS and USS categories were used for the study. The USS cough syrup samples were delivered to parent's volunteers for a period of 3 months and re-evaluated on submission.

Organoleptic Assessment

The organoleptic properties such as colour, odour and taste were undertaken for both sample categories using the appropriate sense organs.

Physicochemical Analysis

pH and viscosity of samples were determined using the Meddler Toledo's pH apparatus (Switzerland) and the U-tube viscometer (Fungilab) respectively. [14] Triplicate determinations were performed and the average value taken for each sample.

Microbiological Analysis

The method of Adeola *et al*. and Razvi *et al.* were adapted for the determination of microbial limit. [10, 15] Samples from USS and UNS categories each were serially diluted and plated on nutrient agar plates in triplicate and were incubated at 30°C for 24 hours to allow for bacterial growth. The ensuing colonies were further purified, isolated and characterized using standard methods. [13]

Antibiotic Susceptibility Testing

Susceptibility tests were performed following modified Kirby-Bauer disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI). [16] In brief, a commercially prepared antibiotic discs containing the following antibiotics; ceftriaxone 30 µg, cloxacillin 5µg, ofloxacin 5µg, erythromycin 5µg, gentamicin 10µg, cefuroxime 30µg, ceftazidime 30µg, amoxicillin/clavulanic acid 20/10µg, nitrofurantoin 300µg, ampicillin 10µg and ciprofloxacin 5µg were

placed equidistance to each other onto sterile Muller Hinton agar seeded with bacteria isolates from paediatric cough syrups. The plates were incubated invertedly at 37°C for 24 hours and the resulting inhibition zone diameter (IZD) interpreted using CLSI protocols. [17]

RESULTS

Organoleptic Properties

The organoleptic assessment is presented in Table 1 and it shows a variation between the samples categories used. The colour changes were from dark brown to colourless, pleasant or offensive odour while taste varied from minty to bland.

Physicochemical Analysis

The result of physicochemical characteristics as presented in Figure 1 shows an increase in pH between sample categories. The pH ranged between 3.13 - 4.82 in UNS categories and 5.00 - 8.34 in USS samples. However, there was a decrease in viscosity from the UNS to USS samples; 4.03 to 9.09 and 0.22 to 4.54 Pascal seconds (Pa.s) respectively (Figure 2).

Microbiological Analysis

Microbial enumeration tests are required to demonstrate production under acceptable hygienic conditions [6] and data obtained are presented in Table 2 and Figure 3. The result revealed a heavy contamination of USS samples with a minimum of four bacterial species each in the following order: *Staphylococcus aureus* (48%) > *Escherichia coli* (22%) > *Pseudomonas aeruginosa* (17%) > *Klebsiella pneumoniae* (13%). A 4 (20%) of the UNS cough syrups screened were contaminated with *Staphylococcus aureus*. The total aerobic viable counts from these contaminated samples ranged between 1.2 - 7.0 × 10^3 cfu/mL and 1.4 - 4.0 × 10^5 cfu/mL in UNS and USS samples respectively. These values exceed USP permissible limit of <10^3 cfu/ml.

Antibiotic Susceptibility Testing

The result of susceptibility profile of isolates recovered from screened paediatric cough syrup samples to the commonly prescribed antibiotics are presented in Figures 4 to 7 and their resistance pattern to antibiotics (Figure 8).

Table 1: Organoleptic Properties of Syrup Categories

Sample Code	Colour		Odour		Taste	
	UNS	USS	UNS	USS	UNS	USS
N1	Dark brown	Pale brown	Caramel	Caramel	Minty	Less Minty
N2	Pale Ox-blood	Pale red	Less Minty	Odourless	Bland	Less Minty
N3	Pale Pink	Pale pink	Offensive	Offensive	Bland	Less Minty
N4	Red	Pale red	Caramel	Caramel	Sour	Sour
N5	Pink	Colourless	Fruity	Less Fruity	Minty	Less Minty
N6	Pink	Pale Pink	Fruity	Less Fruity	Minty	Less Minty
N7	Red	Pale red	No distinct odor	No distinct odour	Bitter-minty	Bitter
N8	Ox-blood	Pale red	Aromatic	Offensive	Minty	Less Minty
N9	Red	Light Red	No distinct odor	No distinct odour	Minty/salty	Salty
N10	Dark-brown	Black	Aromatic	Offensive	Bitter-minty	Bitter
N11	Caramel	Caramel	Fruity	Less Fruity	Mixed fruit/menthol	Less Fruity
N12	Deep-pink	Light pink	Aromatic	Offensive	Fruity	Less Fruity
N13	Brick-red	Pale red	Aromatic	Offensive	Minty	Less Minty
N14	Pink	Pale pink	Almond fruit odor	Less fruity	Mild-sweet	Mildly sweet
N15	Pink	Pale pink	No distinct odor	No distinct odour	Slightly-sweet	Bland
N16	Burnt-chocolate	Black	Aromatic	Offensive	Minty-sweet	Bland
N17	Burnt-chocolate	Brown	Pleasant	Unpleasant	Minty, slightly sweet	Bland
N18	Deep-pink	Light pink	Fruity	Less Fruity	Minty-sweet	Bland
N19	Pale Pink	Light pink	Offensive	Less Fruity	Bland	Mildly sweet
N20	Colourless	Colourless	Nil	Offensive	Less Sweet	Sour

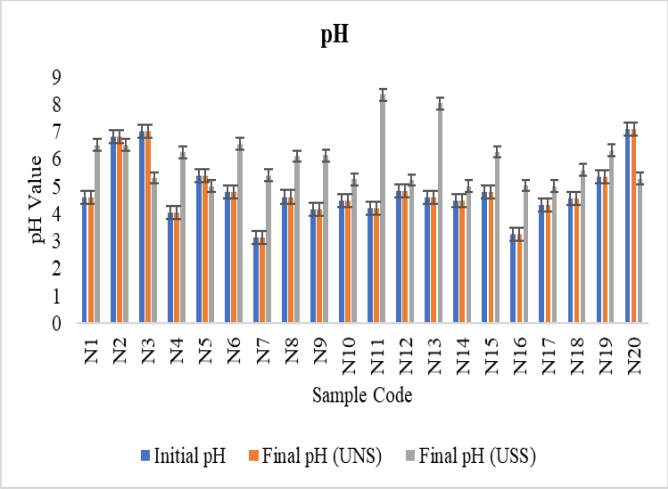

Fig. 1: pH of paediatric cough syrup categories

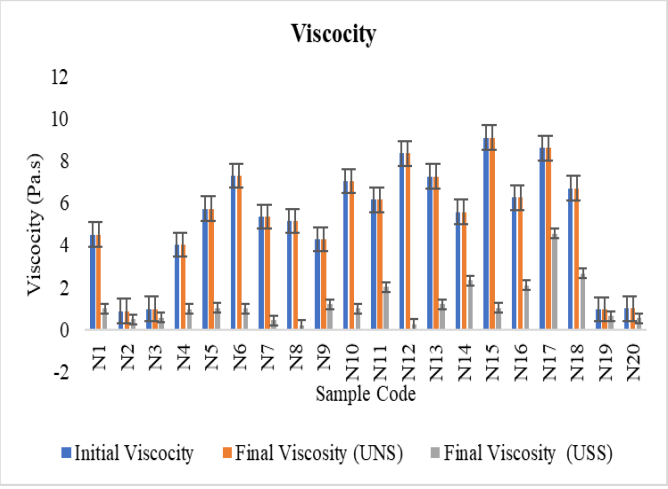

Fig. 2: Viscosity of paediatric cough syrup categories

Table 2: Total viable counts from samples

Sample Code	Total viable counts (cfu/mL)		
	Initial	Final (UNS)	Final (USS)
N1	Nil	Nil	2.4×10^5
N2	7.5×10^3	7.0×10^3	1.6×10^5
N3	3.0×10^3	2.1×10^3	2.8×10^5
N4	Nil	Nil	1.8×10^5
N5	Nil	Nil	2.8×10^5
N6	Nil	Nil	3.5×10^5
N7	Nil	Nil	1.4×10^5
N8	Nil	Nil	1.9×10^5
N9	Nil	Nil	1.6×10^5
N10	Nil	Nil	3.1×10^5
N11	Nil	Nil	3.5×10^5
N12	Nil	Nil	1.6×10^5
N13	Nil	Nil	3.3×10^5
N14	Nil	Nil	2.2×10^5
N15	Nil	Nil	1.4×10^5
N16	Nil	Nil	2.0×10^5
N17	Nil	Nil	3.1×10^5
N18	Nil	Nil	1.5×10^5
N19	1.1×10^3	1.3×10^3	1.8×10^5
N20	1.6×10^3	1.2×10^3	4.0×10^5

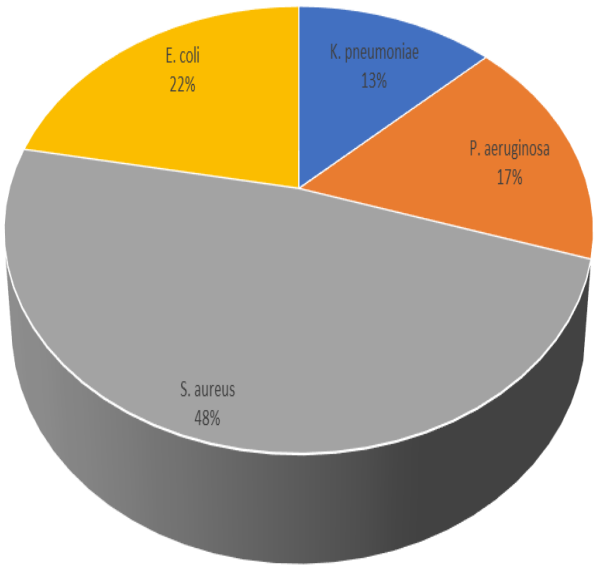

Fig. 3: Prevalence of microbial isolates in paediatric cough syrups screened

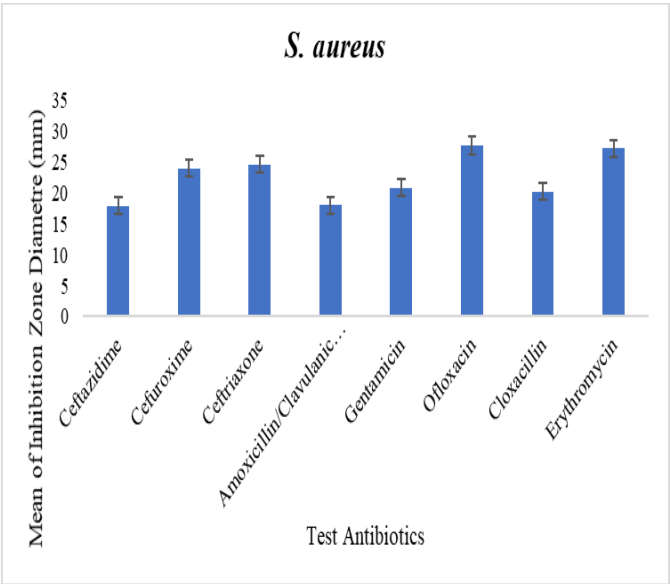

Fig. 4: Antibiogram summary for *Staphylococcus aureus*.

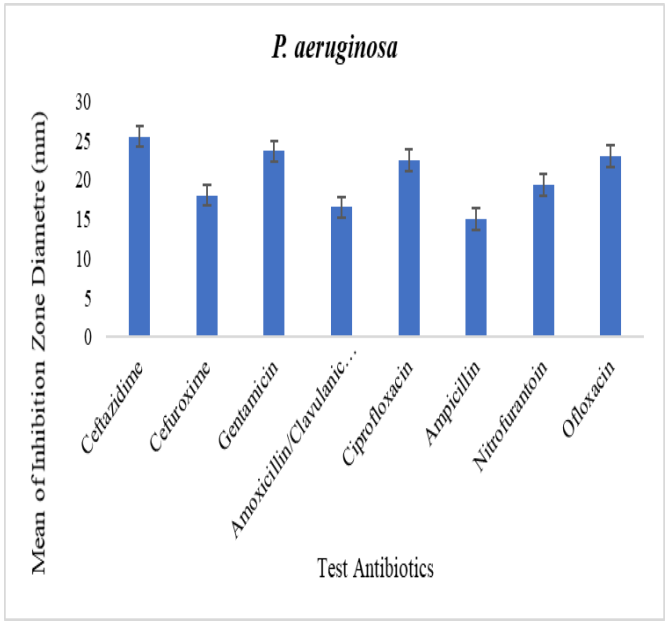

Fig. 5: Antibiogram summary for *Pseudomonas aeruginosa*

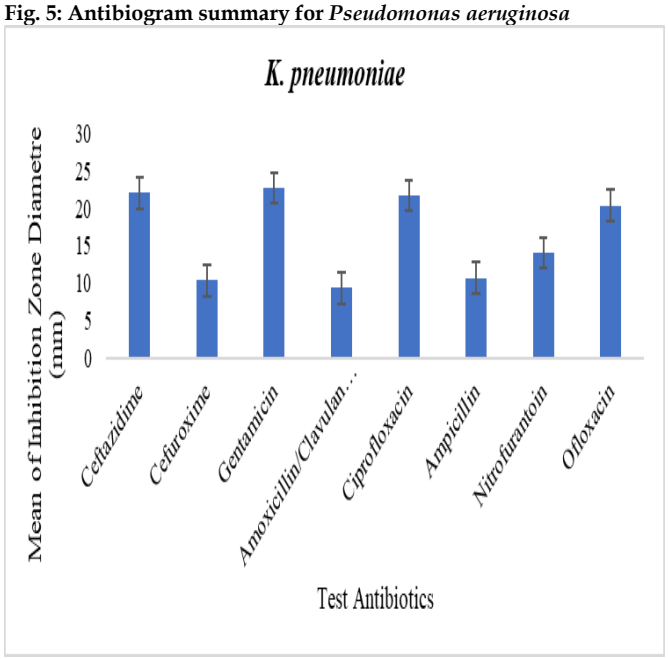

Fig. 6: Antibiogram summary for *Klebsiella pneumoniae.*

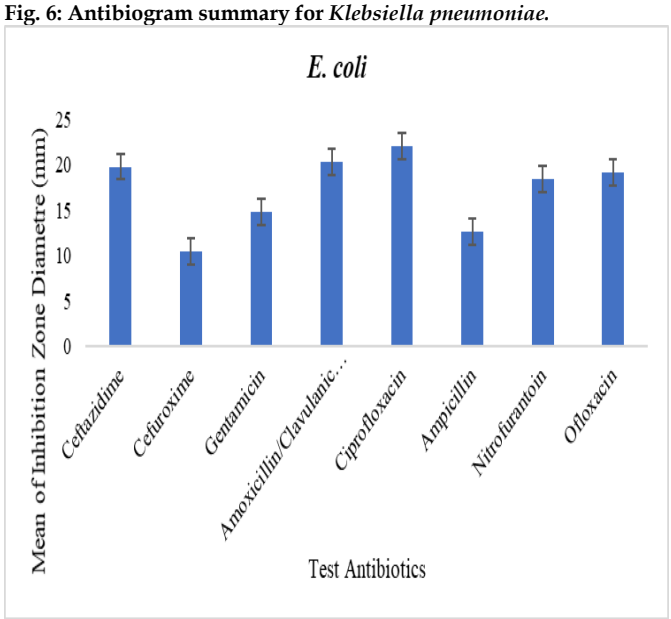

Fig. 7: Antibiogram summary for *Escherichia coli.*

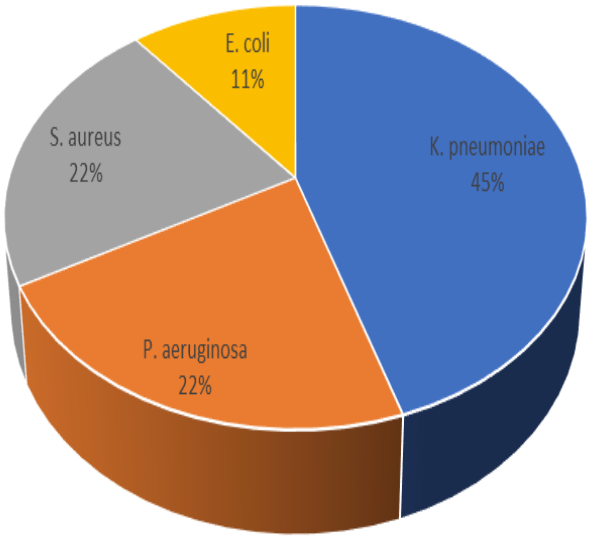

Fig. 8: The prevalent resistance to antibiotics.

DISCUSSION

The present study has demonstrated that microorganisms are widespread in nature. 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 guidelines such as US and European pharmacopoeia. [6-7]

The results of the study confirm the presence of viable and potentially pathogenic microorganisms, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia* in paediatric cough syrups in south-south Nigeria. These findings are consistent with those of previous studies by Mugoyela and Mwambete on microbial contamination of non-sterile pharmaceuticals in public hospital settings. [4] Introduction of microbial contaminants into pharmaceutical products has been known to affect the desirable characteristics of the formulation such as the physicochemical and therapeutic properties. [18] These being encountered during production, storage and in-use, can result from water, other raw materials, environment, excipients and personnel. [6-7] The observed organoleptic changes, pH and viscosity in the products strongly suggest the presence of contaminants (microbe) which results in fermentation, turbidity as previously reported. [19] While acidity prevents microbial attack, [20] some excipients in the formulation are ready substrates for microbial growth. [20] However, previous authors have shown that at neutrality, bacterial spoilage is more likely, with pseudomonads and related Gram-negative bacteria growing in antacid mixtures, flavoured mouthwashes and distilled or demineralized water. [20]

Although heavy contamination by *Staphylococcus aureus* was expected, it is part of the normal body flora just like *K. pneumoniae* but they have the propensity of causing skin infections, pneumonia, meningitis, destructive changes to the lungs and bacteremia [21-22] whereas presence of *E. coli* indicates faecal contamination arising from raw materials including water, *P. aeruginosa* in the soil, water and on the skin can cause inflammation, sepsis and pneumonia. These conditions nevertheless are more worrisome to the paediatrics with poorly developed physiology and immune system.

Contamination seen in the USS sample category may have arisen from personnel factors including poor education, improper drug handling, hygiene and sanitation. Storage temperature notably in the tropics could cause sufficient condensation of water vapour which leads to the dilution of the syrup at its surface [4] hence the decreased viscosity recorded. The warm humid conditions that are characteristic of a tropical environment like Port Harcourt, located in the south-south region of Nigeria have been reported to be more conducive for the growth of various organisms. [4]

The result of antibiotic susceptibility testing of recovered isolate showed microbial resistance to the commonly prescribed antibiotics. Resistant of *Klebsiella pneumoniae* to one antibiotic in three antibiotic categories (Penicillin or β-lactamase inhibitors and Furan antibiotics) indicated multi-drug resistance whereas *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* are extensive-drug resistant since them are resistant to one antibiotic in two antibiotic categories. [23] These menace if not curbed can lead to loss in antibiotic use and efficacy.

In conclusion, this study has demonstrated the need for continuous quality assessment of pharmaceutical products especially for the paediatrics and possible transferance of antibiotic resistance microbial strain through these products. This calls for more stringent measures during product manufacturing, packaging, distribution as well as effective post-marketing surveillance. Consumers should be made aware of proper handling and storage procedure in order to maintain the microbiological quality of their medication.

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