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ANTIMICROBIAL SUSCEPTIBILITY OF COMMON PATHOGENS ISOLATED FROM PAEDIATRIC COUGH SYRUPS IN PORT HARCOURT METROPOLIS, NIGERIA

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ABSTRACT

The presence of unwanted microbes or their metabolites in pharmaceutical products especially syrups are rapidly becoming a matter of public health concern worldwide. Patients being treated with these contaminated syrups can have secondary infection from the pathogenic microorganisms which may complicate the treatment procedure since syrups are mostly administered to children whose immune systems are not well developed. Hence this study was conducted to determine the susceptibility of common pathogens isolated from cough syrups sold in Port Harcourt metropolis. A total of fifty cough syrups were randomly purchased from approved pharmacy outlets within Port Harcourt metropolis and subjected to macroscopic assessment, isolation, characterization, pH, stability as well as susceptibility-resistance test of isolates using standard techniques. The result indicated that 70% of the cough syrup did not show growth while 30% of the syrups were contaminated with microorganisms which were above the acceptable limit ($<10^2$ and $<10^1$ CFU/mL for bacteria and fungi respectively) as specified by the USP. The major contaminants in the cough syrup were *Staphylococcus aureus* (35.29%), *Pseudomonas aeruginosa* (29.43%), *Escherichia coli* (11.76%), *Candida albicans* (11.76%), *Klebsiella pneumoniae* (5.88%) and *Proteus* species (5.88%). The pH value of the cough syrups ranged from 2.76 to 7.40, hence, 96% of the syrups were acidic. For the stability test, 4 out of the 50 samples failed the test. The antibiotic susceptibility testing showed that the isolates had single and multiple drug resistance patterns to the antibiotics used, hence the existence of pathogenic microorganisms in the syrups poses serious health problem to the consumers. A routine microbiological study of these drugs is thus suggested along with good manufacturing practice.

KEYWORDS: Antimicrobial, Susceptibility, Contamination, Pathogens, Paediatric, Cough Syrup.

INTRODUCTION

Each year children develop all sorts of respiratory tract related infections as compared with adults.[1] The most common symptoms are sore throat, fatigue, irritability, loss of appetite and cough. In children, the condition can be distressing, impacting negatively on their sleep, performance and ability to engage in recreational activities.[2] Not only that it can, cause considerable parental anxiety, disturb other family members' sleep, cause trouble for school teachers and carers.[2] Coughing in particular, is a reflex action, it is the body's way of responding to irritants that enters the throat or airways, this irritant stimulates sensory nerves that sends message to the brain.[1] The management of chronic coughing relates to first making an accurate underlying diagnosis and then applying specific treatment for that condition.[2] For most children, the cough will have resolved itself in days, but if it persist for several weeks or one that brings up discolored or bloody mucus may call for medical attention.[1,3] On the advice of a health professional, over-the-counter cough syrups may be prescribed.

Syrups are popular non-sterile dosage forms for administering active medicaments to babies, children and elderly since tablets cannot be administered conveniently.[4] It's a mixture of sugar and water which forms a thick sticky solution that is often flavoured or medicated or concentrated with juice of fruit, plant and often susceptible to contamination by a variety of microorganisms during manufacturing and consumption.[5,6] Also in diluted form, syrups are ready substrates for microbial growth and requires the addition of preservatives.[7] Industrially formulated syrups often contain ingredients to improve solubility, stability, taste or appearance which also increase product vulnerability.[6,7] Contamination of these preparations with potentially pathogenic organisms constitutes serious threat to children because their immune system is poorly developed as previously reported.[7] The most serious problems of contamination as previously reported is where there are no obvious signs of spoilage.[5,8] Although spoilage does not necessarily depend upon the growth of these contaminants but may be facilitated if the formulation and the ambient conditions of

temperature and humidity encourage their multiplication.[9] When these criteria are satisfied changes in the product will occur and may ultimately manifest themselves to the user in several ways. These may include physical deterioration of the product, conversion of formulation into toxic metabolites, reduce therapeutic activity of the product.[8,10] Such product(s) as previously described would be microbiologically unsafe for consumption, a potential health hazards to patients, constitute wastage and may as well yield serious economic losses to the manufacturers.[5] Clinical hazards that are attributable to microbiologically contaminated pharmaceuticals have been published by a number of authors.[8,11-13] Microorganisms that have been isolated from contaminated cough syrups include species of *Streptococcus*, *Staphylococcus*, *Bacillus*, *Micrococcus*, *Enterococcus*, *Clostridium*, *Escherichia*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Aspergillus*, *Candida*, yeast and moulds,[6,8] with microbial count exceeding acceptable limits.[8,14] A great majority of these undesirable organisms are reported to have been introduced through raw materials, processing plant, packaging materials, operatives or elsewhere in the environment during product manufacturing, storage or use.[6,9] In particular, water is the most common raw material in pharmaceutical manufacturing because of its use in rinsing, cleaning of equipment, floors and walls.[15] Although, the survival of these organisms in the environment are sometimes influenced by the presence of relatively inert materials. Thus, microbes can be more resistant to heat or desiccation in the presence of starch, acacia or gelatin.[9] Since microorganisms are widespread in nature and their ability to adapt and survive under a variety of conditions poses a serious risk to biological products. An understanding of the microbial entry points and implementation of measures to prevent microbial contamination is critical for manufacture of safe, pure ad potent biologic products as previously reported.[16] Consequently, this study was undertaken to examine some of the cough syrups sold in pharmacy and patent medicine outlet within Port Harcourt metropolis, isolate and identify any microbial contaminants and carry out antibiotic susceptibility test to ascertain their safety for use.

MATERIALS AND METHODS

Sample collection

A total of 50 different brands of children cough syrups were randomly purchased from approved pharmacy outlets within Port Harcourt metropolis. All samples were physically examined for batch numbers, manufacturing/expiry dates and National Agency for Food and Drug Administration and Control (NAFDAC) Registration Numbers.

Determination of physicochemical parameters

pH: This was determined using the method described by Okpo *et al*.[5] with some modification. The tip (approximately 4cm) of pH meter (Mettler Toledo's, Wincom Company Limited, China) was immerse in the syrup (10 mL volume of each syrup already dispensed into a sterile 20 mL beaker) and stir gently for few seconds. For faster response and to avoid cross contamination of the samples, the electrode tip was rinse with the syrup to be tested, before taking measurements. pH meter reading was taken, when the display becomes stable.

Stability testing: Stability testing of the cough syrup was performed by keeping the samples at accelerated temperature conditions. A portion of each cough syrup was taken in universal bottles and monitored at room temperature. The samples were assessed for turbidity, change in colour, odour and homogeneity at the interval of 24 hours, 3, 7 and 14 days.[17]

Standardization of culture

The standardization of inoculums for susceptibility testing was carried out by adjusting the turbidity of the microbial suspension to match the turbidity of 0.5 McFarland Standards. This is equivalent to approximately 1.5 x 10^8 CFU/mL of bacterial suspension, when the turbidity values of the two suspensions match optically or visually.

Isolation of microbial contaminants

Tests for microbial contaminants were conducted on all fifty samples and the isolated microbes were subjected to macroscopic examination and other typical growth characteristics on selective, non-selective, differential culture media and complemented. From the growth obtained on the different culture media, morphologically different isolated colonies were separately streaked for purification onto the surface of Nutrient Agar plates for bacteria and Sabouraud Dextrose Agar plates for fungi. The isolated colonies were then gram stained and identified by conventional microbiological (biochemical and physiological) tests.[11,18-20]

Antimicrobial susceptibility tests for the isolates

Isolated microbial contaminants were tested for their susceptibility to the commonly used antibiotics following the modified Kirby-Bauer disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) using Muller Hinton agar.[21] Discs containing the following antibacterial agents were used: Amoxicillin/Clavulanate 30 µg, Ofloxacin 5 µg, Cloxacillin 5 µg, Erythromycin 5 µg, Ceftriaxone 30 µg, Gentamicin 10 µg, Cefuroxime 30 µg, Ceftazidime 30 µg against gram positive bacteria and Amoxicillin/Clavulanate 30 µg, Ofloxacin 5 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, Cefuroxime 30 µg, Ceftazidime 30 µg, Ampicillin 10 µg, Nitrofurantoin 300 µg against gram negative bacteria. For the fungal isolates, fluconazole (25 mg), ketoconazole (20 mg) and nystatin (25 mg) were used. An uninoculated plates containing only the media and antibiotic disc were used as blank to compare the different samples. The plates were incubated at 37^{o}C for 24 hours (bacteria) and 22^{o}C for 7 days (fungi) after which the zones of inhibition

were measured in millimeter and the mean calculated for each antimicrobial agent. Using the interpretative chart derived from the zones of inhibition of standard organisms according to the Clinical Laboratory Science Institute (CLSI), the zone size of each antimicrobial agent was interpreted and the isolate was reported as being "resistant", "intermediate" or "susceptible".[22]

RESULTS AND DISCUSSION

Physicochemical examination (pH and stability test)

Physical-chemical examination conducted on the fifty commercially available cough syrups revealed the following data shown in Table 1.

Table 1: pH and stability test results of the 50-cough syrup examined.

Sample Code	pH	Stability Test*	Sample Code	pH	Stability Test*
C1	4.31	No change	C26	7.40	- Presence of particles, - Change of colour from light pink to cream - Foul smell.
C2	5.04	No change	C27	6.08	No change
C3	5.66	No change	C28	5.25	No change
C4	4.35	No change	C29	3.85	No change
C5	5.78	No change	C30	5.28	No change
C6	5.15	No change	C31	6.18	No change
C7	6.23	No change	C32	5.80	No change
C8	3.82	No change	C33	5.20	No change
C9	5.48	No change	C34	5.23	No change
C10	5.86	No change	C35	5.34	No change
C11	5.15	No change	C36	7.10	No change
C12	6.89	No change	C37	4.48	No change
C13	5.80	No change	C38	4.08	No change
C14	5.14	No change	C39	5.30	No change
C15	5.48	No change	C40	3.76	No change
C16	3.87	No change	C41	4.51	No change
C17	4.97	- Pink to dark brown	C42	2.76	No change
C18	4.92	- Presence of dark particles, foul smell	C43	4.33	No change
C19	5.22	No change	C44	4.50	No change
C20	6.71	No change	C45	3.72	No change
C21	5.83	No change	C46	6.01	No change
C22	5.55	No change	C47	3.83	No change
C23	7.21	- Light pink to brown	C48	4.40	No change
C24	6.23	No change	C49	3.75	No change
C25	4.85	No change	C50	3.95	No change

*After 3 weeks at room temperature

From the result, the pH of the cough syrup samples was within the range of 2.76 to 7.40, three of the cough syrup samples had alkaline pH of 7.10 – 7.40 while the rest of the syrups had acidic values of less than 7. A good number of the cough syrups are known to contain ingredients that can maintain or impact pH levels. For example, sodium citrate and citric acids are commonly used as flavours. Citric acid is especially erosive because of its acidic nature and the ability to chelate calcium at higher pH. Most of the cough syrups investigated contain citrate which may therefore be partly responsible for the generally low pH levels show. Aqueous ammonium chloride solution is mildly acidic and used in cough syrup preparation. Its expectorant action is caused by its irritating action on the bronchial mucosa. This causes the production of excess respiratory fluid which presumably is easier to cough up, but its contribution to low pH and acidity of cough syrups is not well established.[23] The pH of any product is one of several important factors that determine the survival and growth of microorganisms during processing, storage and distribution. Non-adherence to good manufacturing practices may lead to decomposition of some ingredients in the formulation, resulting in pH change and ultimately, microbial contaminations.[11] As a result, manufacturers should be interested in determining the pH of their product viz as viz maintaining pH at certain levels to control microbial growth and prevent product deterioration and spoilage.

For the stability test, four out of fifty samples recorded change in colour, smell including the presence of particle after 21 days under room temperature. The indication is that high tropical temperature affects the stability of cough syrups marketed in Port Harcourt and possibly leads to the degradation of the products long before the manufacturers' stated expiry dates.[24] Other factors that may have influenced stability includes chemical, physical properties of the active substance,

pharmaceutical excipients, dosage form, its composition, manufacturing process, nature of the container-closure system, and the properties of the packaging materials.[17] Generally, stability testing is to provide evidence on how the quality of an active substance or pharmaceutical product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light.[17]

Microbiological analysis

In this study, fifty samples were examined for microbial contamination, and the results obtained (Figure 1, Table 2) shows 15 out of the 50 samples being contaminated with a minimum of six isolates in the following order: *Staphylococcus aureus* (35.29%) > *Pseudomonas aeruginosa* (29.43%) > *Escherichia coli* (11.76%) > *Candida albicans* (11.76%) > *Klebsiella pneumonia* (5.88%) > *Proteus spp* (5.88%). The result of the microbial limit test ranged from 3.00 x10^3 CFU/mL to 4.40 x 10^{11} CFU/mL for bacteria and 9.50 x 10^5 to 6.20 x 10^7 CFU/mL for fungal isolates (Table 2).

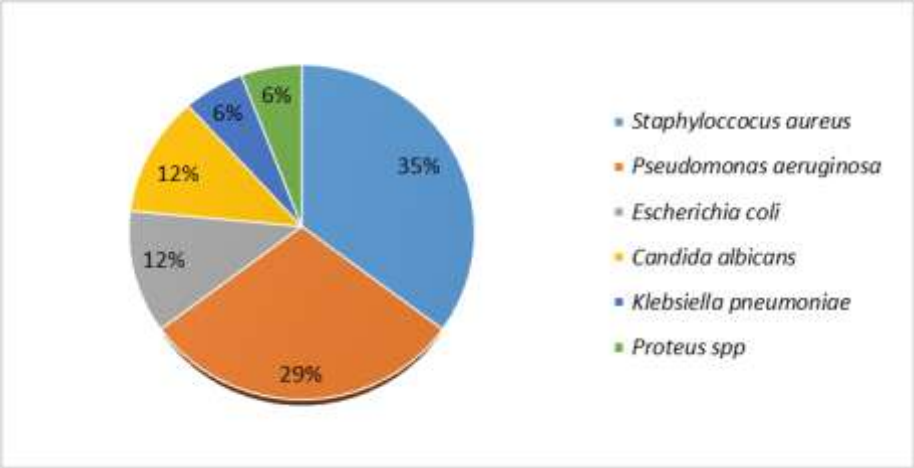

Fig. 1: Percentage distribution of isolated organisms.

Table 2: Total Viable Cell Count of Contaminated Cough Syrup Samples.

Sample Code	CFU/mL	
	Bacterial count	Fungal count
C3	3.0 x 10^3	NG
C17	NG	9.5 x 10^5
C20	4.2 x 10^4	NG
C22	5.3 x 10^3	NG
C23	5.0 x 10^4	NG
C24	3.0 x 10^4	NG
C25	6.0 x 10^{10}	NG
C26	4.4 x 10^{11}	NG
C27	4.6 x 10^3	NG
C35	NG	6.2 x 10^7
C39	3.3 x 10^4	NG
C42	3.4 x 10^3	NG
C47	5.0 x 10^3	NG
C48	4.9 x 10^5	NG
C49	8.0 x 10^3	NG

*NG = No growth

Contamination of non-sterile pharmaceutical liquid products by microorganisms has long been a major concern when discussing public health risks and drug efficacy. Not only is the presence of these organisms harmful, but also the presence of toxins released by these organisms.[8] The degree of the risk will vary from product to product and patient to patient, depending on the types and numbers of organisms present, the route of administration, and the resistance of the patient to infection.[25] Therefore, non-sterile preparations must pass microbial bioburden tests and tests for the absence of certain specified indicator pathogens. Results obtained indicates that 70% of the syrup samples analysed showed microbial growth within acceptable limit of < 10^2 CFU/mL (bacteria) and 10^1 CFU/mL (fungi) as specified in the USP for microbial contamination of syrups.

However, the microbial load of the remaining 15 samples were far higher than the acceptable limit. Although sterility is not a requirement in the official compendia for non-sterile pharmaceutical products, the microbial load of such product should be within the acceptable limit.[8] In accordance with these requirements, the findings of this study show the presence of microbial contamination in 15 samples tested, thus rendering them unfit for the intended purposes. This seems to be is a common problem which has been reported for several non-sterile medicaments.[8,26-29] Irrespective of whether they are harmful or objectionable or nonpathogenic, can bring about changes in the physical characteristics, including the breaking of emulsions, thinning of creams, appearance of turbidity or deposit, off ordour and colour changes.[30] In turn, these changes not only make the product aesthetically unacceptable but can also affect the therapeutic potency and dosage delivery.[25]

One of the findings of great concern is the presence of viable and potentially pathogenic microorganisms, namely, *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*.[31,32] The microbial contamination may be attributable to poor adherence to GMP, quality control procedures and inadequate post-marketing surveillance of medicines and other medical appliances. The presence of *S. aureus* as a contaminant reflects contamination of processing unit and/or raw material.[27] The organisms, primarily inhabits the skin and mucous membranes of warm-blooded vertebrates and may have been introduced during handling and processing by personnel.[33] Previous report shows that *Staphylococcus species* can survive in fairly high concentration of sugar, even though favourable growth of microorganism should not occur in the presence of high sugar concentration.[27] Although *Staphylococcus* species are easily killed during heating, their toxins are known to be resistant and so not easily destroyed by heat.[34] Product at highest risk of transmitting *Staphylococcus* toxins are those that are not properly sterilized. The presence of *P. aeruginosa*, *K. pneumonia* and *Proteus spp* suggest contamination from raw materials used as well as the conditions prevalent in the environment in which the products are manufactured and packaged,[27] The presence of *E. coli* is an indicator organism on test samples for fecal contamination as they are not always confined to the intestine and able to survive for brief period outside of the body.[35] The occurrence of *E. coli* is source of great concern with respect to hygienic practices, lack of adequate handling of the products and suggests the route of contamination is possibly water.[27] The presence of *C. albicans* can cause secondary infection in immune-compromised patients (HIV/AIDS patients) especially when present in large numbers. Hence, the need take care, regardless of the species as they could easily cause increased allergies and toxicity.[36]

In common with other studies,[27,28,34] none of the tested samples showed presence of *Salmonella* spp, whereas the most microbial contaminants in this study was *S. aureus*. This could be explained by the fact that these bacteria can maintain cellular homeostasis while enduring environmental challenges, such as changes in host cell core temperature or exposure to phagocyte-mediated reactive oxygen species.[37] Their presence in product suggests poor environmental hygiene during processing or badly contaminated or adulterated raw materials as previously described.[8]

Antimicrobial susceptibility testing

The results presented in the figures reveal that *S. aureus* was susceptible to gentamicin, ofloxacin, it showed varied susceptibility to ceftriaxone, cefuroxime, erythromycin and was resistant to ceftazidime, augmentin (amoxicillin/clavulanate) and cloxacillin (Fig. 2). *P. aeruginosa* was susceptible to gentamicin, ofloxacin, ceftazidime, ciprofloxacin and showed varied susceptibility to augmentin, nitrofurantoin, and ampicillin, it was resistant to cefuroxime (Fig. 3). *E. coli* was susceptible to gentamicin, ofloxacin, nitrofurantoin, augmentin (amoxicillin/clavulanate) but showed varied susceptibility to ceftazidime, ciprofloxacin and was resistant to ampicillin and cefuroxime (Fig. 4). *K. pneumonia* was susceptible to gentamicin, ofloxacin and nitrofurantoin; it showed varied susceptibility on ciprofloxacin and was resistant to ceftazidime, cefuroxime, augmentin (amoxicillin/clavulanate) and ampicillin (Fig. 5). *Proteus* spp was susceptible to gentamicin, ofloxacin, ceftazidime, it showed varied susceptibility to ciprofloxacin, augmentin (amoxicillin/clavulanate) and was resistant to cefuroxime, nitrofurantoin and ampicillin (Fig. 6). For *C. albicans* susceptible (S) was recorded against nystatin only (Fig. 7).

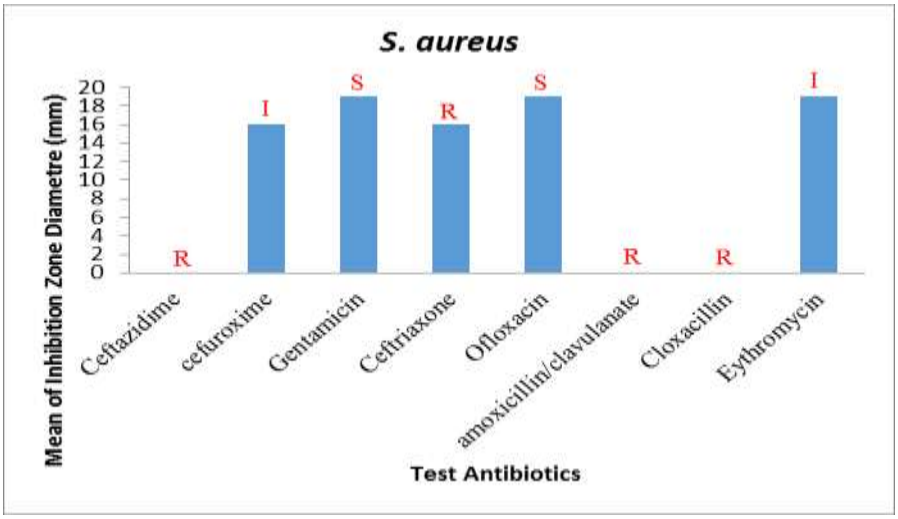

Fig. 2: Antibiotic susceptibility test result for *Staphylococcus aureus*. The result shows that *S. aureus* was susceptible (S) to Gentamicin, Ofloxacin and resistant (R) to Cloxacillin, Ceftazidime and Augmentin (Amoxicillin/Clavulanate) while Cefuroxime, Ceftriaxone and Erythromycin showed intermediate (I) effect.

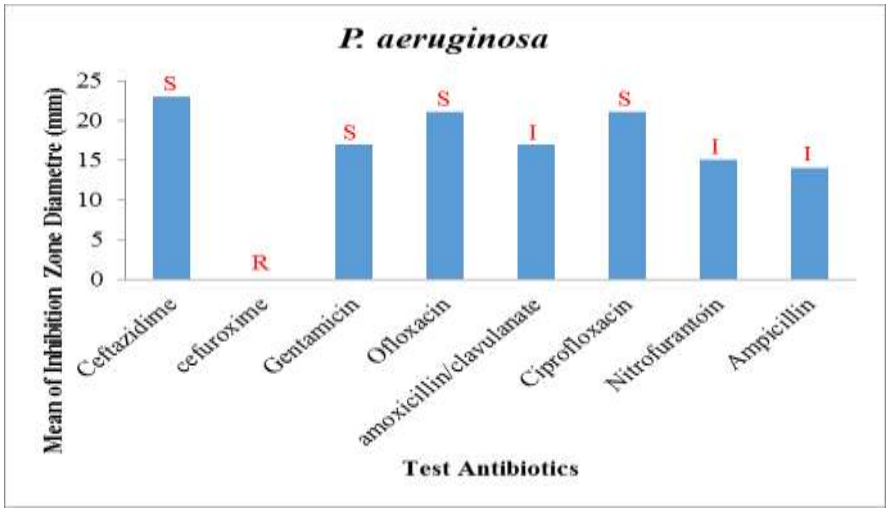

Fig. 3: Antibiotic susceptibility test result for *Pseudomonas aeruginosa*. The result shows that *Pseudomonas aeruginosa* was susceptible (S) to Ceftazidime, Gentamicin, Ofloxacin, and Ciprofloxacin and resistant (R) to Cefuroxime, while Augmentin (Amoxicillin/Clavulanate), Nitrofurantoin and Ampicillin showed intermediate (I) effect.

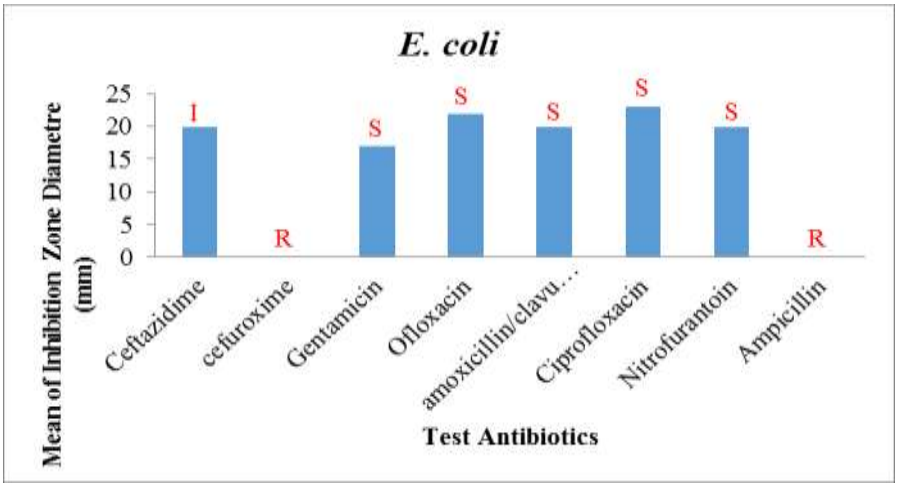

Fig. 4: Antibiotic susceptibility test result for *Escherichia coli*. From the result *E. coli* was susceptible (S) to Gentamicin, Ofloxacin, Augmentin (Amoxicillin/Clavulanate), Ciprofloxacin, Nitrofurantoin and resistant (R) to Cefuroxime, and Ampicillin but showed intermediate (I) effect with Ceftazidime.

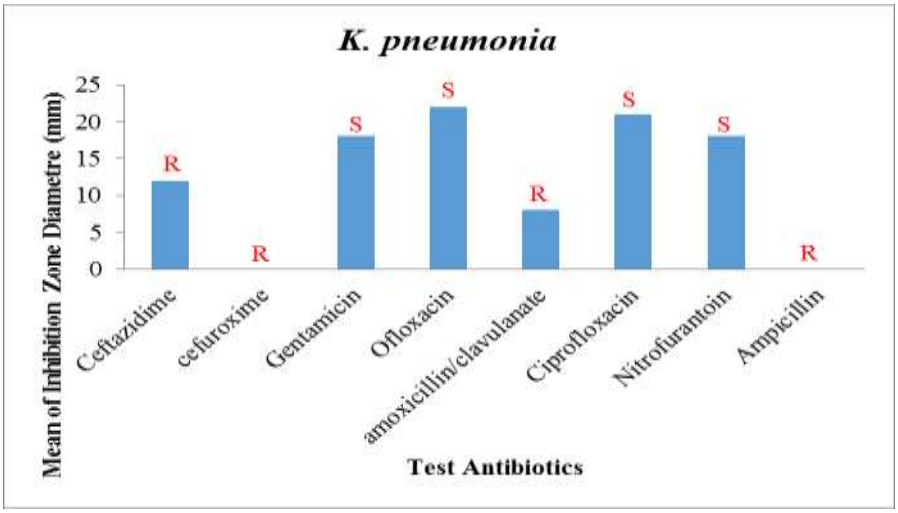

Fig. 5: Antibiotic susceptibility test result for *Klebsiella pneumonia*. From the result for *K. pneumonia* was susceptible (S) to Gentamicin, Ofloxacin, Nitrofurantoin, Ciprofloxacin and resistant (R) to Cefuroxime, Augmentin (Amoxicillin/Clavulanate), Ceftazidime and Ampicillin but no intermediate (I) effect.

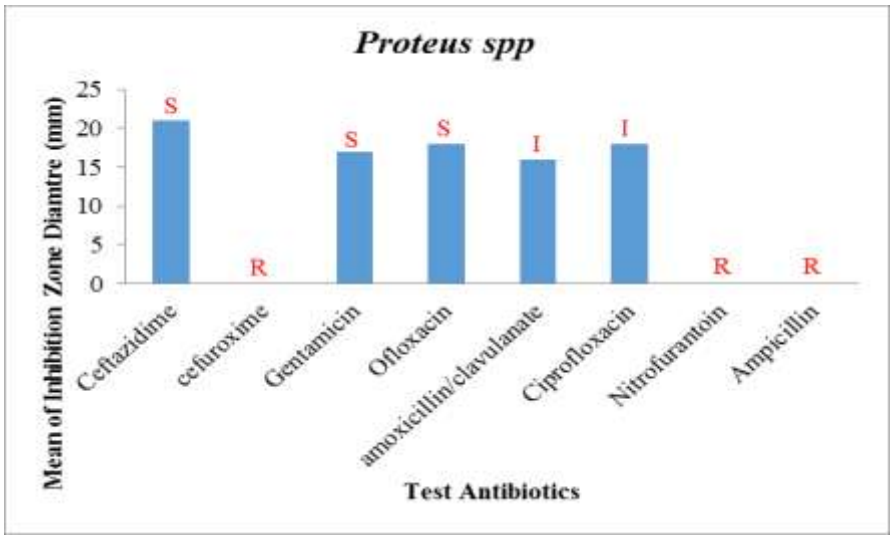

Fig. 6: Antibiotic susceptibility test result for *Proteus* spp. Results indicates susceptibility (S) to Gentamicin, Ofloxacin, Ceftazidime but resistant (R) to Cefuroxime, Nitrofurantoin, Ampicillin and showing intermediate (I) effect with Ciprofloxacin and Augmentin (Amoxicillin/Clavulanate).

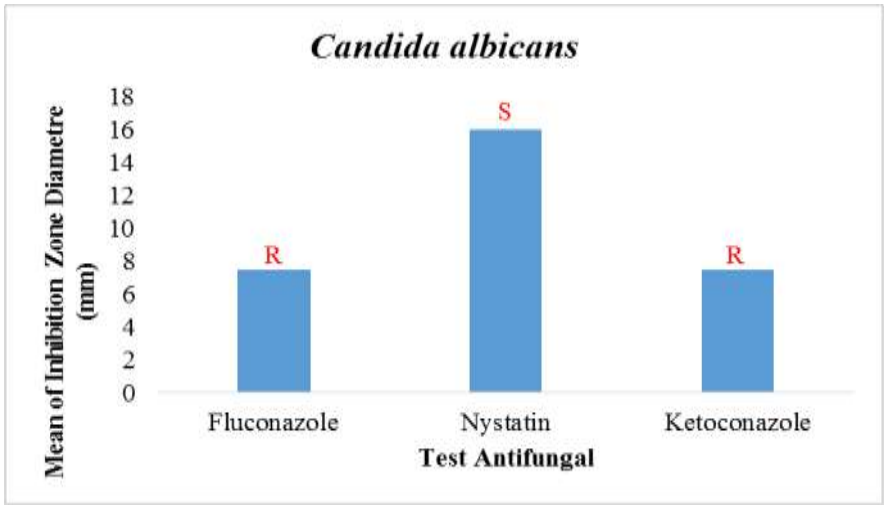

Fig. 7: Antifungal susceptibility test result for *Candida albicans*. The fungal isolate was susceptible (S) to nystatin but resistant (R) to fluconazole and ketoconazole.

Percentage resistance of microbial isolates

Also, the results in Table 3 reveal the percentage resistance of organisms to antibiotics tested. For bacteria, *K. pneumonia* had the highest percentage resistance of 50% followed by *S. aureus* (37.5%), *Proteus spp* (37.5%), *E. coli* (25%), *P. aeruginosa* (12.5%), while that of *C. albicans* was 66.7%.

Table 3: Percentage Resistance of Test Isolates.

Isolates	Resistance (%)	Resistant to
Bacteria		
Klebsiella pneumonia	50.0	Cefuroxime, Augmentin, Ceftazidime, Ampicillin.
Staphylococcus aureus	37.5	Cloxacillin, Ceftazidime, Augmentin.
Proteus spp	37.5	Cefuroxime, Nitrofurantoin, Ampicillin
Escherichia coli	25.0	Cefuroxime, Ampicillin.
Pseudomonas aeruginosa	12.5	Cefuroxime
Fungi		
Candida albicans	66.7	Fluconazole, Ketoconazole

Antimicrobial susceptibility tests are important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual isolates.[38] Although, empirical therapy may be still effective for some pathogens because resistance mechanisms have not yet been established. Results reveal that *K. pneumoniae* was more resistance (50%) to the antibiotics used, followed by *S. aureus* and *Proteus* spp (37.5%), *E. coli* (25%) and the least was *P. aeruginosa* (12.5%). The level of fungal contamination in this study was less than that of bacteria (Table 2), however it was observed that fungal resistant was 66. 7% higher than that of bacteria because of the number of antifungals involved. Overall, the percentage of resistant in the present study is close to that previously reported in similar studies, where incidence of contamination of varying degrees were reported.[4,6,8,39] Acquired resistance to the antimicrobial agents tested may have arisen from a combination of several mechanisms including change in permeability target, overexpression of efflux systems, resistance by mutations of the topoisomerase in the quinolone-resistance determining region, condition of growth (acidity and alkaline environments of growth), composition of medium.[7,40] Globally, nonsusceptibility of microorganisms to commonly used antimicrobial agents is rapidly increasing with variations occurring temporally and regionally.[41,42] Empirical therapy seems to be based on epidemiological data that are updated and adapted geographically.[43] Accordingly, it is necessary to take note of the resistance patterns of each region in order to implement appropriate control measures as well as developing rational policies for each use.[40]

K. pneumoniae isolates are known to be naturally resistant to amoxicillin and ampicillin, due to a constitutively expressed chromosomal class-A β-lactamase.[40,44] Varying occurrence rates to other antibiotics have been reported by several authors.[4,40,44] Resistance of *S. aureus* to beta lactamase antibiotics such as cloxacillin, augmentin and ceftazidime may be due to the fact that *S. aureus* produces the enzyme penicillinase which inhibits the effect of these antibiotics.[45] Ampicillin, amoxicillin, and narrow-spectrum cephalosporins are known to be labile strong inducers for the *Proteus* species.[44] Ureidopenicillins and carboxypenicillins, cefotaxime, and ceftriaxone are labile weak inducers and so remain active against inducible strains, although resistance or decreased susceptibility is evident in derepressed mutants.[44] *E. coli* present the simplest cases, as they usually have only insignificant levels of uninducible molecular class C enzymes, often called AmpC types.[44] Previous report indicates that they are inherently susceptible to ampicillin and the narrow-spectrum cephalosporins, such as cephalothin and cephalexin whereas resistance is typically apparent only to those agents that penetrate poorly, such as isoxazolyl penicillins and benzylpenicillin.[44] Resistance of *P. aeruginosa* may have developed either through the acquisition of resistance genes on mobile genetic elements (i.e., plasmids) or through mutational processes that alter the expression and/or function of chromosomally encoded mechanisms. Both strategies for developing drug resistance are reported to severely limit the therapeutic options for treatment of serious infections.[46] Our finding on the resistance of *C. albicans* to antifungals, such as fluconazole is in agreement with previous studies.[47] Goldman *et al*.[48]; Brion *et al*.[49]; Ribeiro & Rodrigues,[50] have shown similar incidences of increasing resistance of *C. albicans* to both systemic and topical antifungal agents and thus making effective treatment more difficult. Consequently, therapeutic alternatives based on different strategies may be required. As previously reported, the presence of potentially pathogenic opportunistic microbes, should not be ignored. The reason being that they may cause a significant deterioration in the health status of the end users, particularly, those who are immunologically compromised, and of infants with an immature immune system.[8]

CONCLUSION

In this study, *Staphylococcus aureus* was the leading microbial contaminants amongst other isolates. Majority of the isolates showed high resistance to cefuroxime, with varying susceptibility patterns to erythromycin, ceftriaxone, augmentin and very highly susceptible to ofloxacin and Gentamicin. The gram-negative organisms were susceptible to nitrofurantoin except *Proteus spp*

that showed varied susceptibility to ciprofloxacin. For bacteria, *Klebsiella pneumonia* showed the highest percentage antibiotic resistance while the least resistant was *Pseudomo*nas *aeruginosa*. The study further demonstrates the importance of maintaining product standards all through the process of manufacturing, packaging, distribution and having effective post-marketing surveillance. Non-compliance at any stage may greatly affect the microbiologic quality of the end products and ultimately the final user of such product.

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