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In-Vitro Comparison of Antimicrobial Actions of Probiotics (*Lactobacilli* Species and *Saccharomyces boulardii*) with Standard Antibiotics for the Treatment of Diarrhea in Pediatric Population

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Abstract

Background and objectives: Irrational and repeated use of broad spectrum antibiotics for infectious diarrhea in children has resulted in their increased resistance along with several systemic toxic effects. Probiotics are also used in the management of infectious diarrhea as these are supposed to be favorable in promoting overall health benefits including stability of the intestinal flora. However, these agents are not used as an alternative to antibiotics as their exact bactericidal/bacteriostatic effects have not been evaluated on the basis of any clinical or *in-vitro* samples (Culture and Sensitivity test). Hence the aim of our study was to compare the culture and sensitivity patterns of standard antibiotics and two probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* used for the treatment of infectious diarrhea in children less than 5 years of age in a tertiary care hospital of Karachi, Pakistan. **Methodology:** This prospective quasi experimental study was conducted for a period of six months. After getting informed consent from parents/guardians, the stool samples were obtained from children of ages, 6 months to 5 years, presented with signs and symptoms of diarrhea in outpatient department (OPD) or being referred to microbiology department for stool C/S (culture and sensitivity). The sensitivity patterns of the cultured isolates were assessed for standard antibiotics according to the CLSI guidelines (2018), while the two probiotics (*Lactobacilli* and *Saccharomyces boulardii*) were evaluated by means of *Dried Modification method*. The data was

analyzed using statistical software SPSS version 19.0. **Results:** A total number of 325 stool samples were collected, out of which 152 samples were positive for pathogens *i.e. E. coli*, *Klebsiella* and *Salmonella typhi.* The sensitivity of combination of *Lactobacilli* for *E. coli*, *Klebsiella* and *Salmonella typhi* was 28.3%, 25% and 25% respectively. While, for *Saccharomyces boulardii* the sensitivity for *E. coli*, *Klebsiella* and *Salmonella typhi* was 37%, 32.1% and 25% respectively, which were slightly higher or equivalent to commonly prescribed antibiotics such as Amoxicillin/Clavulanic acid, Ceftazidime, Ampicillin, Cefotaxime, Cefuroxime, Ceftriaxone, Aztreonam, Trimethoprim/Sulfmethoxazole and Nalidixic acid. In comparison, the antibiotics which are not frequently used for infectious diarrhea showed higher sensitivities for all isolated organisms; as for *E. coli* the highest sensitivity was observed for Amikacin (96.7%), Gentamycin (95.7%) Imipenim (95.7%) and Piperacillin/Tazobactam (84.8%). Moreover, for *Klebsiella* the highest sensitivity was observed for Imipenim (98.2%), followed by Amikacin (94.6%), Piperacillin/Tazobactam (92.9%) and Gentamycin (89.3%). **Conclusion:** On *in-vitro* cultured samples, the two probiotics *Lactobacilli* and *Saccharomyces boulardii* have shown slightly higher or equivalent sensitivity in comparison to the most commonly prescribed antibiotics (Amoxicillin/Clavulanic acid, Ceftazidime, Ampicillin, Cefotaxime, Cefuroxime Ceftriaxone, Aztreonam, Trimethoprim/Sulfmethoxazole and Nalidixic acid). However, both probiotics displayed lower sensitivity in comparison to some broad spectrum but less commonly prescribed antibiotics (Amikacin, Gentamycin, Imipenim and Piperacillin/Tazobactam) in our clinical settings.

Keywords

Antibiotics, Probiotics, *Lactobacillus paracasei*/*Lactobacillus acidophilus*, *Saccharomyces boulardii*, *in Vitro*

1. Introduction

Diarrhea is one of the most common infectious diseases among humans globally [1]. It causes significant health risk particularly among pediatrics with most vulnerable age group affected is children less than 5 years of age, which is also, accounted for the high mortality rates in this age group [2]. ORS (oral rehydrating solution) has been a mainstay treatment in managing 90% of children with mild to moderate diarrhea [3]. Along with ORS zinc supplements are also found helpful in reducing the duration and volume of stools [4]. However, in pediatric infectious diarrhea antibiotics including Ampicillin, Cefexime, Ceftriaxone, Amikacin, Nalidixic acid and Ciprofloxacin are also required as their immunity is not sufficient to clear the infections [5]. Antibiotics on account of their toxicity, have limited use in pediatric age group [6] as well as their resistance is a rising threat to the human worldwide these days [7].

In Pakistan irrational and repeated use of antibiotics for infectious diarrhea in

children below 5 years of age has resulted in increased resistance and several toxicities of broad spectrum antibiotics, including alteration of the normal gut flora; which could lead to potential risk for future serious infections among children [8].

Evidence from the literature suggested the clinical use of probiotics for the treatment of bacterial gastroenteritis [9]. Probiotics are defined as the living microorganisms which when dispensed in the body at appropriate amounts prove to be favorable in promoting the health benefits, and at the same time it also increases the stability of the intestinal flora [10]. The probiotics used for the treatment of diarrhea in children are of bacterial or fungal origin. The bacterial strains of commonly used probiotics are *Bifidobacterium* and *Lactobacillus*, whereas, *Saccharomyces boulardii* is a yeast, which is a type of fungus [11]. The strains of *Lactobacilli* and *Saccharomyces boulardii* have proven its efficacy in reducing diarrhea [12].

Currently the probiotics are used along with antibiotics for infectious diarrhea but their direct bactericidal/bacteriostatic effects have not been tested or proved on the basis of any laboratory data or *in-vitro* culture and sensitivity tests. To the best of our knowledge, the current study is innovative across the globe. This study will help us to know the efficacy of probiotics *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* in comparison to the standard antibiotics against microbial organisms causing infectious diarrhea in children less than 5 years of age on the basis of stool culture and sensitivity.

2. Material and Methods

This prospective quasi experimental study was carried out in a pediatric unit and the microbiology laboratory of a tertiary care hospital, Dr. Ruth. K. M. Pfau Civil Hospital Karachi from December 2017 till May, 2018. The eligibility criteria for the recruitment in this clinical study were; children aged six months to five years, clinical diagnosis of acute diarrhea as per World Health Organization (WHO) criteria (*i.e.* having at least four liquid stools in the past 24 hours along with clinical signs and symptoms of dehydration on clinical examination). Children having systemic infection, malabsorption syndrome, severe acute malnutrition, blood in stool, have received antibiotics in last fourteen days or immediately require antibiotic for current infection were excluded.

The research was conducted following the ethical guidelines of Helenski declaration and Pakistan Medical and Research Council. Written informed consent was obtained from the parents or guardian prior to the recruitment of children with diagnosis of acute diarrhea. Moreover, the guardian or parents were comprehensively briefed about the research purpose and procedures involved. Importantly, the anonymity and confidentiality of the study participant's data was maintained throughout the research with no unauthorized person having access to the data. The research is approved by the institutional Ethical Review Com-

mittee (ERC), and the research was initiated after the approval was granted by the ERC.

Parents or guardian were informed and briefed to collect at least 5 gram of faeces of eligible study participants in a sterilized stool culture bottle. The data related to basic demographics was also collected for each eligible participants being recruited in this research.

Routine laboratory examination and stool culture were performed within four hours after collection of specimen. Initially, the stool samples were grossly examined for color and consistency. Later, the microscopic examinations of stool samples were performed to identify the presence of any cellular elements (*i.e.* red blood cells, white blood cells, pus cells), eggs, protozoa, cysts of parasites etc.

The stool culture was performed to identify the enteric pathogen causing acute diarrhea *i.e. E. coli* and *Klebsiella.* Standard procedures and steps (*i.e.* collecting in sterilized container and immediate processing within four hours) were followed for the stool culture process. At day 1 the stool sample was inoculated with *Salmonella Shigella* agar (SS agar), MacConkeys agar, and selenite enrichment broth being incubated aerobically at 37˚C overnight. Following day, subculture from selenite F broth on *Salmonella Shigella* agar was performed. At day 3, the stool cultures were re-examined for the presence of organisms *i.e. E. coli*, *Klebsiella and Salmonella.* The biochemical identification was later done for the confirmation of pathogenic strain by using; Simon citrate agar, SIM medium agar, Urea agar and TSI agar. The serological analysis was carried out by using *E.coli* and *Salmonella* and *Shigella* Antisera.

On each of the stool sample that showed positive growth of *E. coli*, *Klebsiella* and *Salmonella*, all standard antibiotics and two probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) *and Saccharomyces boulardii* were applied. The standard antibiotics applied were Amikacin, Amoxicillin, Gentamycin, Cefuroxime, Ceftazidime, Aztreonam, Ampicillin, Cefotaxime, Ciprofloxacin, Nalidixic Acid, Levofloxacin, Ceftriaxone, Imipenim, Trimethoprim-Sulfamethoxazole, Cefoperazone/Sulbactam and Piperacillin/Tazobactam, according to the Clinical Laboratory Science Institute (CLSI) 2018 guidelines [13]. The Zones of Inhibition (ZOI) of each antibiotic were measured. The calibrated vernier caliper was used to measure the diameters in millimeters (mm) of each antibiotic disc along with the clear surrounding clear area till the edges of the clear zone (showing no bacterial growth).

While the two probiotics, *Lactobacilli* and *Saccharomyces boulardii* were analyzed by dried modification method [14]. Standard procedure and protocol were followed where initially 2 µL of overnight culture was spotted on MRS agar. Later plates were dried at room temperature for half an hour and incubated an aerobically on 37˚C for 18 hours. Plates were overlaid with 10 ml of specific microorganism specific medium. Once the colonies were developed, the plates were again overlaid with 10 ml of soft microorganism specific medium and later laid for overnight culture of the target pathogenic strain. Following, 48 hours of in-

cubation the ZOI was measured and interpreted as (ZOI > 20 mm as sensitive and less than 10 mm as resistant).

3. Statistical Analysis

The data was analyzed using statistical software SPSS version 19.0. Initially, the data was validated twice for incorrect entries by checking with the study proforma. The categorical variables (*i.e.* gender, age categories and culture isolates) were presented as frequency/percentage while for their significance Chi square was applied.

4. Results

In the present research, stool sample from 325 children with confirmed diagnosis of acute diarrhea were collected and among those, 152 stool samples showed positive bacterial growth. Figure 1 gives details of the age categories (months) of 152 children with positive stool culture. Among all 152 positive culture isolates majority, 53 (34.9%) were found to be in the age group of 13 - 24 months, around eight percent in less than or equal to 12 months and 37 - 48 months of age categories. Figure 2 gives details of gender distribution. Majority, 57% were males while forty three 43% percent were females.

Table 1 shows the mean ZOI (mm) for individual organisms isolated from the stool samples of children with diarrhea for all antibiotics and the two probiotics *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii.*

Figures 3-5 show the sensitivity pattern of different antibiotics and Probiotics, *Lactobacilli* and *Saccharomyces boulardii* for different organisms isolated. For *E. coli* the highest sensitivity was observed for Amikacin (96.7%), Gentamycin

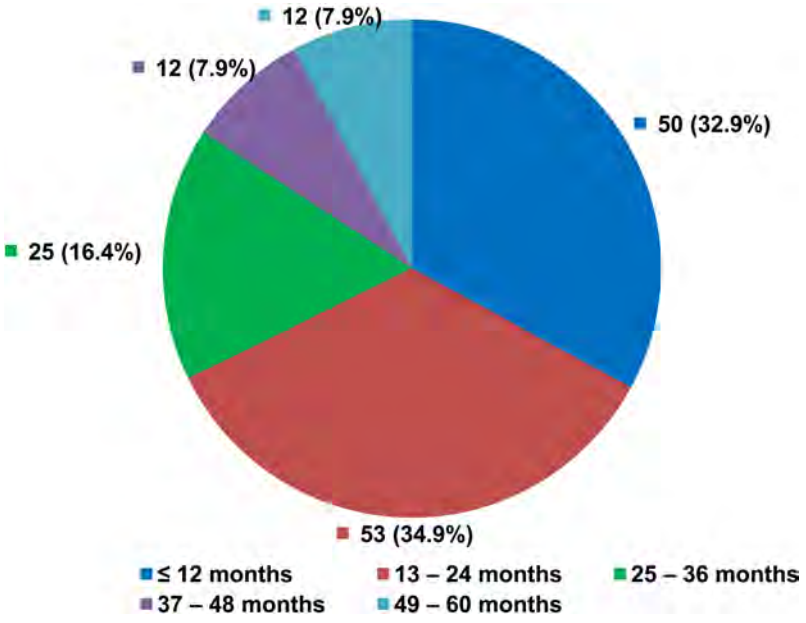


Figure 1. Age Distribution (n = 152).

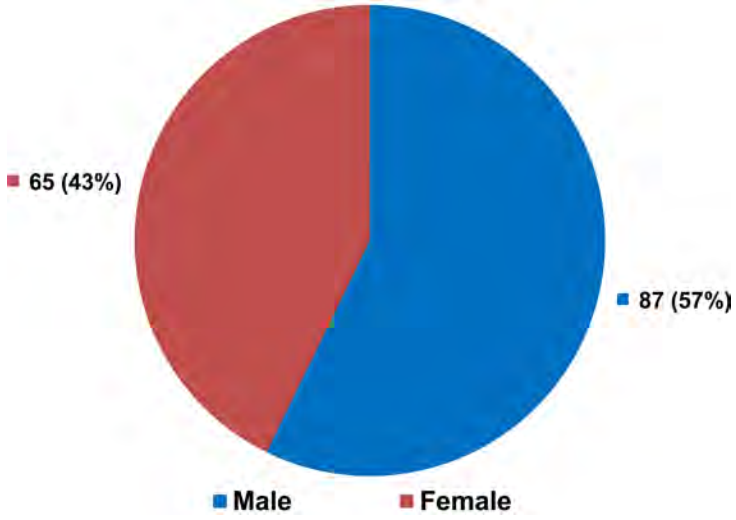
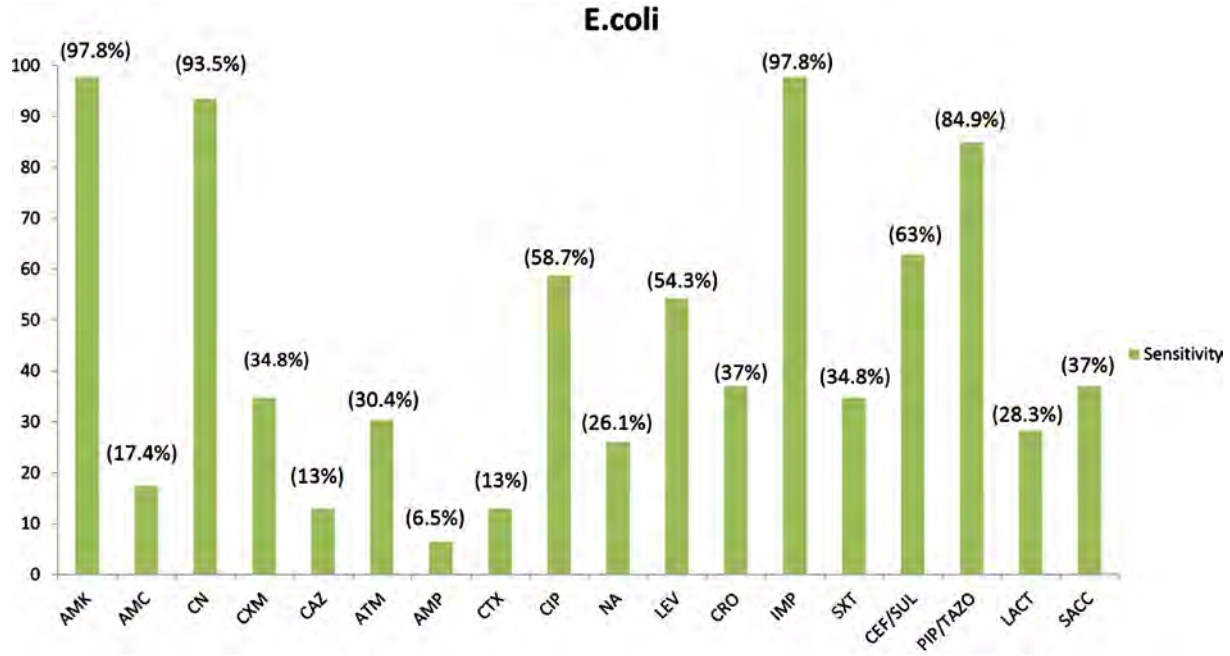


Figure 2. Gender Distribution.

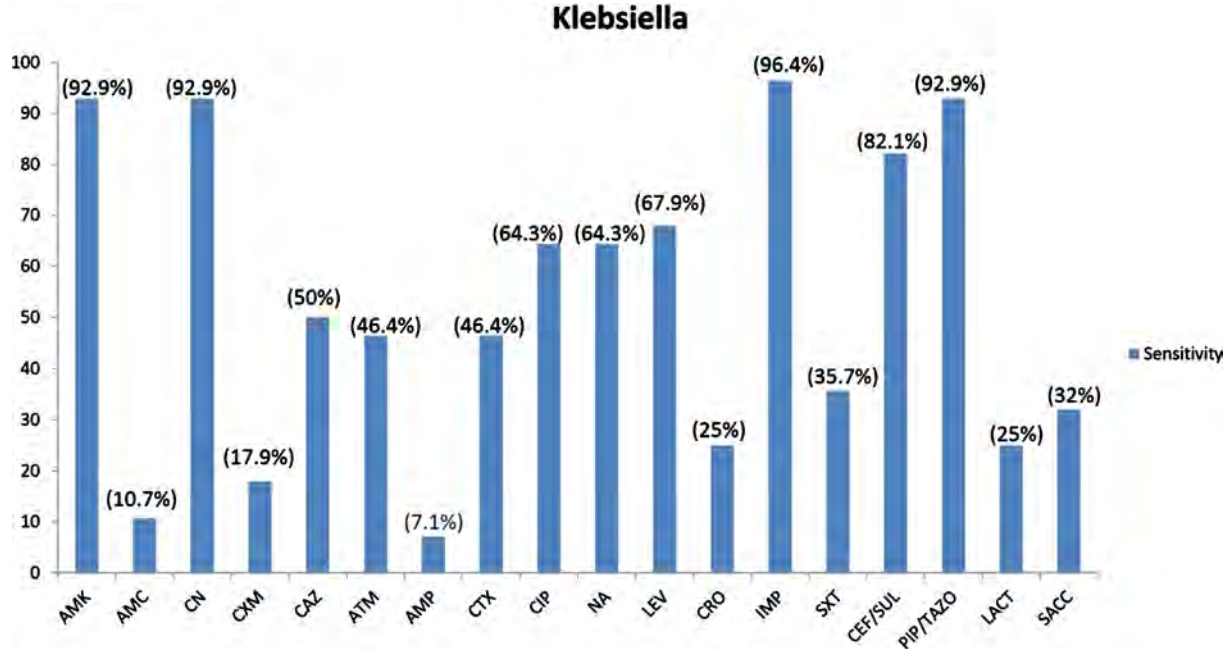
Table 1. Mean Zones of Inhibition (mm) of antibiotics and probiotics *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* for all three Organisms isolated from the stool samples of children with diarrhea, total N = 152.

Antibiotics and Probiotics	*E. coli*	*Klebsiella*	*Salmonella typhi*
	Z.O.I (mm) Mean ± SD	Z.O.I (mm) Mean ± SD	Z.O.I (mm) Mean ± SD
Amikacin	18.78 ± 1.90	18.75 ± 1.73	18.25 ± 0.96
Amoxicillin	12.18 ± 3.59	11.64 ± 3.22	11.75 ± 0.96
Gentamycin	16.48 ± 1.76	16.50 ± 1.95	17.00 ± 1.83
Cefuroxime	14.64 ± 4.07	13.46 ± 3.81	13.50 ± 4.51
Ceftazidime	16.60 ± 3.49	18.00 ± 3.76	15.75 ± 1.50
Aztreonam	17.43 ± 3.91	18.25 ± 3.79	16.50 ± 3.42
Ampicillin	11.84 ± 1.82	12.27 ± 1.86	12.50 ± 0.58
Cefotaxime	21.18 ± 3.09	23.27 ± 4.03	19.50 ± 0.58
Ciprofloxacin	18.62 ± 4.79	19.14 ± 4.94	23.25 ± 0.50
Nalidixic Acid	14.55 ± 4.57	16.20 ± 4.58	13.25 ± 4.57
Levofloxacin	15.49 ± 3.90	15.86 ± 3.87	18.00 ± 1.16
Ceftriaxone	20.67 ± 4.26	20.13 ± 4.20	19.00 ± 3.37
Imipenim	23.57 ± 1.74	23.86 ± 1.59	23.25 ± 4.27
Trimethoprim-Sulfmethoxazole	11.16 ± 4.74	11.16 ± 5.27	10.25 ± 5.32
Cefoperazone/Sulbactam	19.03 ± 4.79	20.25 ± 4.38	19.25 ± 5.56
Piperacillin/Tazobactam	21.62 ± 2.90	21.71 ± 2.43	22.25 ± 2.06
Saccharomyces boulardii	11.58 ± 4.23	10.64 ± 3.65	9.25 ± 1.71
Lactobacilli (*Lactobacillus paracasei*/*Lactobacillus acidophilus*)	9.86 ± 3.24	9.24 ± 3.	9.25 ± 1.71



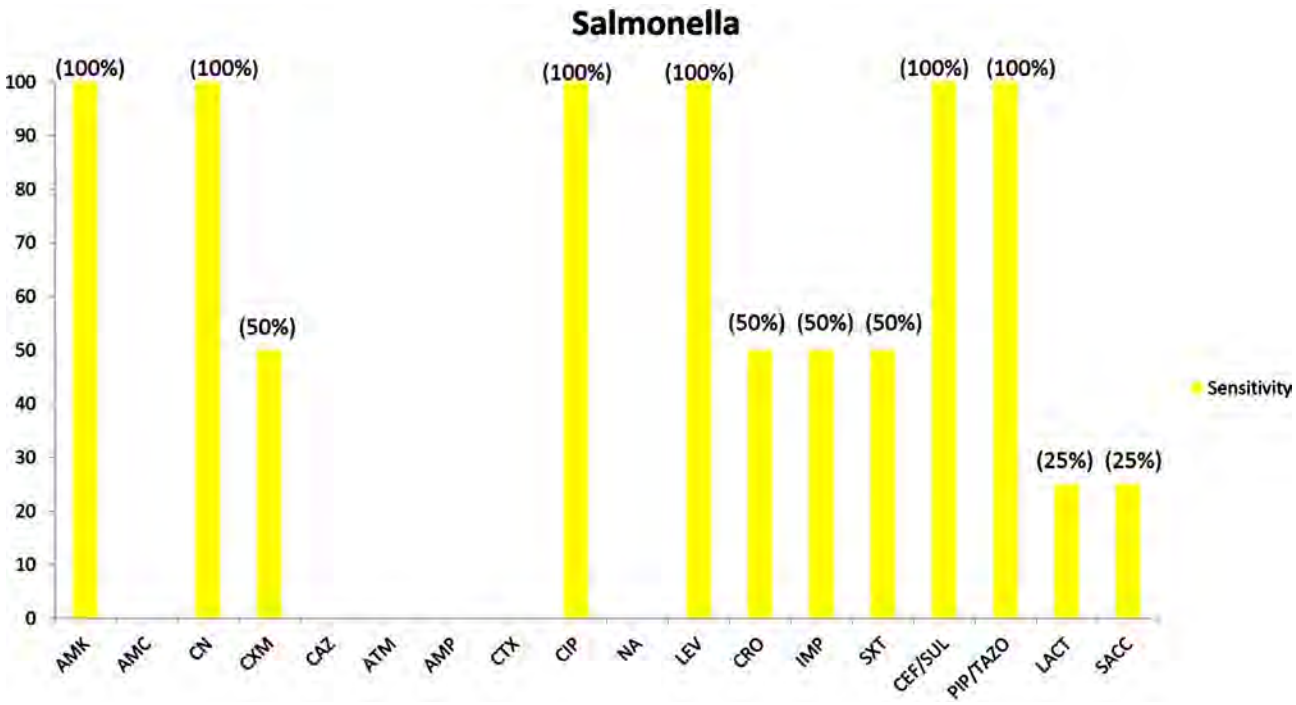
AMK; Amikacin, AMC; Amoxicillin-clavulanic acid, CN; Gentamycin, CXM; Cefuroxime, CAZ; Ceftazidime, ATM; Aztreonam, AMP; ampicillin, CTX; cefotaxime, CIP; Ciprofloxacin, NA; Nalidixic acid, LEV; Levofloxacin, CRO; Ceftriaxone, IMP; Imipenem, SXT; Trimethoprim-Sulfamethoxazole, CEF/SUL; Cefoperazone/Sulbactam, PIP/TAZO; Piperacillin/tazobactam, LACT; *Lactobacillus paracasei*/*Lactobacillus acidophilus*, SACC; *Saccharomyces boulardii.*

Figure 3. Sensitivity patterns of Antibiotics and Probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* for *E. coli* (n = 92).



AMK; Amikacin, AMC; Amoxicillin-clavulanic acid, CN; Gentamycin, CXM; Cefuroxime, CAZ; Ceftazidime, ATM; Aztreonam, AMP; ampicillin, CTX; cefotaxime, CIP; Ciprofloxacin, NA; Nalidixic acid, LEV; Levofloxacin, CRO; Ceftriaxone, IMP; Imipenem, SXT; Trimethoprim-Sulfamethoxazole, CEF/SUL; Cefoperazone/Sulbactam, PIP/TAZO; Piperacillin/tazobactam, LACT; *Lactobacillus paracasei*/*Lactobacillus acidophilus*, SACC; *Saccharomyces boulardii.*

Figure 4. Sensitivity patterns of Antibiotics and Probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* for *Klebsiella* (n = 56).



AMK; Amikacin, AMC; Amoxicillin-clavulanic acid, CN; Gentamycin, CXM; Cefuroxime, CAZ; Ceftazidime, ATM; Aztreonam, AMP; ampicillin, CTX; cefotaxime, CIP; Ciprofloxacin, NA; Nalidixic acid, LEV; Levofloxacin, CRO; Ceftriaxone, IMP; Imipenem, SXT; Trimethoprim-Sulfamethoxazole, CEF/SUL; Cefoperazone/Sulbactam, PIP/TAZO; Piperacillin/tazobactam, LACT; *Lactobacillus paracasei*/*Lactobacillus acidophilus*, SACC; *Saccharomyces boulardii.*

Figure 5. Sensitivity patterns of Antibiotics and Probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* for *Salmonella typhi* (n = 4)

(95.7%) Imipenim (95.7%) and Piperacillin/Tazobactam (84.8%). While, the other antibiotics which showed lower sensitivity are Amoxicillin/Clavulanic acid (17.4%), Ceftazidime (13%), Ampicillin (6.5%), Cefotaxime (13%) and Nalidixic acid (26.1%). Moreover, for *Klebsiella* the highest sensitivity was observed for Imipenim (98.2%), Amikacin (94.6%), Piperacillin/Tazobactam (92.9%) and Gentamycin (89.3%). Although, lower sensitivity is observed for Amoxicillin/Clavulanic acid (10.7%), Cefuroxime (17.9%), Ampicillin (7.1%) and Ceftriaxone (25%), for *Salmonella typhi* the highest sensitivity (*i.e.* 100%) was observed for Amikacin, Gentamycin, Ciprofloxacin, Levofloxacin, Cefoperazone/Sulbactam and Piperacillin/Tazobactam. Whereas, some of the antibiotics such as Cefuroxime, Ceftriaxone, Imipenim, Trimethoprim/Sulfmethoxazole revealed moderate sensitivity (*i.e.* 50%). Few of the antibiotics had shown no sensitivity. However, for *Lactobacilli* the sensitivity for *E. coli*, *Klebsiella* and *Salmonella typhi* were 28.3%, 25% and 25% respectively. While for the second probiotic, *Saccharomyces boulardii* the sensitivity for *E. coli*, *Klebsiella* and *Salmonella typhi* were 37%, 32.1% and 25% respectively.

5. Discussion

Though, different antibiotics have been used for the treatment of bacterial diarrhea, but considering growing resistance of antibiotics and harmful effects, [7]

researchers are more focused towards exploring the alternative means of treatment. Probiotics were first explored in 1907 by a Russian scientist Ellie Metchnikoff who linked the wellbeing and longevity of Bulgarian workers with the substantial ingestion of yogurt which contained large amounts of *Lactobacillus* species [15]. The other probiotic *Saccharomyces boulardii* was discovered in 1920 by a French microbiologist Henri Boulard, during a visit to Indochina, where he found that some people did not develop infectious diarrhea during the outbreak because they were already consuming a special type of tea made up of the outer skin of the tropical fruits lychee and mangosteens [9]. Generally, probiotics are preferred for the treatment of acute diarrhea by clinicians [8] and have been hypothesized in promoting the health benefits including the stability of the intestinal flora [16].

The possible mechanisms of action of *Saccharomyces boulardii* comprises, *in vivo* antimicrobial activity, immune system activation, antitoxin activity which helps to reduce the enterotoxin, enhances the enzymatic activity and favors the absorption and nutrition [17]. Locally, it has shown to secrete a heat-labile factor which decreases bacterial adherence [18]. One of the previous studies also revealed the immunomodulating effects on the intestine of the rats orally treated with *Saccharomyces boulardii*, attributed to increase the levels of secretory IgA and components of crypt cells of the small intestine [19]. Moreover, the probable mechanism of action of *Lactobacillus* species is to increase the mucin expression in human intestinal epithelial cells which blocks the invasion and adherence of pathogenic *E. coli* [20]. Additionally it is able to inhibit tumor necrosis factor (TNF) mediated inflammation and apoptosis in intestinal epithelial cells [21]. It has demonstrated mitogenic effects and has enhanced regeneration of mucosal lining [22]. However their direct antimicrobial effects are largely unknown due to lack of scientific evidence on the basis of *in-vitro* or culture and sensitivity tests. Hence their role as alternatives to antibiotics in bacterial gastroenteritis is not very much convincing as a single agent and used along with antibiotics.

The current study was aimed to evaluate the direct antimicrobial effects of probiotics on the basis of sensitivity testing of cultured stool samples of pediatric patients with diarrhea. This is evident from **Table 1** that probiotics, *Lactobacilli and Saccharomyces boulardii* had shown overall low zone of inhibitions for all organisms in comparison to majority of antibiotics. However, the zones of inhibition of *Saccharomyces boulardii for E. coli* were equivalent to trimethoprim/sulfamethoxazole and ampicillin, 11.16± 4.74 mm and 11.84 ± 1.82 mm respectively. No parallel or comparable data is available reporting the ZOIs for probiotics as our study is the first to assess these on isolated cultured samples of stool in pediatric patients with infectious diarrhea. The results of our study highlighted that the sensitivity of *Lactobacilli* for *E. coli*, was 28.3%, which is higher than majority of the commonly prescribed antibiotics including Amoxicillin/Clavulanic acid (17.4%), Ceftazidime (13%), Ampicillin (6.5%), Cefotaxime

(13%) and Nalidixic acid (26.1%). Similarly, for *Klebsiella* the sensitivity of *Lactobacilli* was 25% and was slightly better in comparison to commonly prescribed antibiotics including Amoxicillin/Clavulanic acid (10.7%), Cefuroxime (17.9%), Ampicillin (7.1%) and was equivalent in comparison to Ceftriaxone (25%).

Whereas, the sensitivity of *Saccharomyces boulardii* for *E. coli*, *was* 37%, slightly higher in contrast to most commonly prescribed antibiotics used for pediatric diarrhea including, Amoxicillin/Clavulanic acid (17.4%), Ceftazidime (13%), Ampicillin (6.5%), Cefotaxime (13%), Nalidixic acid (26.1%), and approximately equivalent to Cefuroxime (34.8%), Aztreonam (30.4%), Trimethoprim/Sulfmethoxazole (34.8%) and Ceftriaxone (37%). The sensitivity of *Saccharomyces boulardii* for *Klebsiella* was displayed to be 32.1%, better than the widely prescribed antibiotics, Amoxicillin/Clavulanic acid (10.7%), Cefuroxime (17.9%) and Ceftriaxone (25%). The lower sensitivity of commonly prescribed antibiotics for *E. coli* and *Klebsiella* in our study revealed that these antibiotics are prescribed irrationally and often misused because of their over the counter availability [23]. However the sensitivity of the two probiotics, *Lactobacilli* (25%) and *Saccharomyces boulardii* (25%) were lower as compared to all antibiotics in case of *Salmonella typhi.*

Furthermore our study also highlighted that two tested probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* had lower sensitivity against all of the three organisms isolated in our stool samples, when compared to more effective antibiotics which are not prescribed routinely for the treatment of infectious diarrhea in adults as well as in pediatrics. Overall for *E. coli*, the highest sensitivity was shown for Amikacin (96.7%), followed by Gentamycin (95.7%) Imipenim (95.7%) and Piperacillin/Tazobactam (84.8%). For *Klebsiella* the highest sensitivity was observed for Imipenim (98.2%), after that Amikacin (94.6%), Piperacillin/Tazobactam (92.9%) and Gentamycin (89.3). Nevertheless the use of majority of the above mentioned antibiotics is limited because of potential toxicities such as nephrotoxicity, ototoxicity, neurotoxicity and elevated levels of sodium, potassium and magnesium. Hence, these antibiotics should not be considered as first-line agents in the treatment of diarrhea in children and should be reserved as a final resort for serious and life-threatening infections. WHO has also warned that frequent use of these highly sensitive antibiotics may result in rise of the resistance of many of the gram negative organisms including *E. coli*, *Klebsiella and Salmonella typhi* [24] [25].

Although several studies have documented the beneficial effects of probiotics in diarrhea but their outcome was on the basis of clinical follow up of the patients with improvement or decrease in the duration of diarrhea. However the magnitude of therapeutic effects of probiotics was neither assessed nor compared with antibiotics, hence no related studies are available.

Besides, a couple of studies revealed that probiotics have become extensively popular and have rapidly achieved high level of use in Europe and Asia for the

cure of diarrhea [26] [27] [28]. The results of a meta-analysis, reported that *Saccharomyces boulardii* significantly reduced the duration of diarrhea (mean difference, −19.7 hours; 95% confidence interval, −26.05 to −13.34), stool frequency on day 2 (mean difference, −0.74; 95% confidence interval, −1.38 to −0.10) and day 3 (mean difference, −1.24; 95% confidence interval, −2.13 to −0.35), the risk for diarrhea on day 3 (risk ratio, 0.41; 95% confidence interval, 0.27 to 0.60) and day 4 (risk ratio, 0.38; 95% confidence interval, 0.24 to 0.59) after intervention compared with control [29].

The National Institute for Health and Clinical Excellence established in England also suggested the use of probiotics along with ORS for the treatment of acute diarrhea in children. The evidence-based clinical practice guidelines based on systematic reviews of randomized controlled trials displayed that *Lactobacilli* and *Saccharomyces boulardii*, were the efficacious agents in reducing the duration of diarrhea by one day [30] [31].

To the best of our knowledge the current study is innovative as for the first time, antimicrobial activity of the two most commonly prescribed probiotics, *Lactobacilli* and *Saccharomyces boulardii* were evaluated and compared with the standard antibiotics used against infectious diarrhea in children on the basis of stool culture and sensitivity. Furthermore the results of the current study also provided the scientific evidence of direct antimicrobial effects of probiotics which is an addition to their already described mechanisms of action. Even though in current study *in-vitro* testing of both probiotics clearly revealed that overall their sensitivities against organisms causing diarrhea were on lower side and not remarkably better in comparison to the majority of the standard antibiotics for infectious diarrhea in our pediatric clinical set up.

6. Conclusions

The results of the present study highlighted that both probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* possess direct antimicrobial or bactericidal action but have overall low sensitivities against microbial organisms causing infectious diarrhea in our clinical set-up. Although both probiotics were found to have either equivalent or slightly higher sensitivities when compared to most commonly prescribed antibiotics (Amoxicillin/Clavulanic acid, Ceftazidime, Ampicillin, Cefotaxime, Cefuroxime, Ceftriaxone Nalidixic acid and Trimethoprim/Sulfmethoxazole). On the contrary, both of the probiotics had lower sensitivities in comparison to more effective and less commonly prescribed antibiotics (Amikacin, Gentamycin, Imipenim and Piperacillin/Tazobactam). Therefore, probiotics cannot be considered as sole agents or alternative treatments to antibiotics and hence antibiotics remain the mainstay of treatment for pediatric infectious diarrhea in our clinical set-ups.

However, the results of current study should be further validated by other multicenter studies conducted on diverse population.

7. Limitations of the Study

The study had certain limitations. Firstly, the study was conducted at only one hospital, where majority of patients are with low socio economic class and low education visits. Secondly, limited sample size and selection of only study site had restricted the generalization and external validity of the study findings. Thirdly, as the study was *in-vitro* where the laboratory analysis was undertaken to compare the sensitivity of antibiotics with probiotics with no follow-up involved.

Future Recommendations

Further similar experimental studies should be carried out on a large sample size in various clinical settings in order to validate the results of current study. Probiotics other than *Lactobacilli* and *Saccharomyces boulardii* should also be assessed and compared with antibiotics and with other probiotics used in the management of pediatric diarrhea.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Pattern of Paediatric Adenoid and Tonsillar Surgery in Ekiti

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Abstract

Background: Surgeries of adenoid, tonsils or both are common pediatric performed by otorhinolaryngologist, head, and neck surgeon worldwide. Clinical pattern and management varied in a different center. This study aimed at determining the rate, socio-demographic features, indications, barriers, types, complications and patients' satisfaction with adenoid and tonsils surgery in low-income countries. **Materials and Methods**: This is a hospital-based retrospective study of pediatric patients who had adenotonsillectomy, adenoidectomy, and tonsillectomy in the study center. This study was carried out over a period of ten years from March 2007 to February 2017. Data for this study was obtained from the medical record department, ENT clinic operation booking register and theatre operation register. All the data obtained were statistically analyzed using SPSS version 16. The data were then expressed by descriptive statistics table, bar charts, and pie charts. Ethical clearance was sought for and obtained from the ethical committee of the institution. **Results**: A total of 463 patients were booked for adenoid and tonsillar surgery out of which 214 patients had surgery done during the study period. This represented 46.2% of the participants that had surgery done. Adenotonsillectomy peaked 38.3% at preschool age group: (1 - 5) years. There were 58.9% males and male to female ratio was 1.5:1. Majority 42.5% of the patients reside in the city while minority 25.7% of the patients were village dwellers. Preschool ages were the majority 40.2% while post-secondary schools ages were the minority 8.4% of the patients. The parents of the majority of the patients were 27.1% health workers and 24.8% business men, while the parent of the minority of the patients was 11.7% farmers and 16.4% industrial workers. Major indications for surgery were 52.3% obstructive sleep apnoea syndrome and 21.5% recurrent tonsillitis. Less common indications for surgery in this study were 1.4% persistent fever and 1.9% cardi-

opulmonary complications. There were 7.0% patients admitted as day cases and 93.0% patients admitted as an in-patient. Postoperatively, 1.4% of the day cases were admitted as inpatients while 1.9% of inpatients were treated as day cases. In this study, the established high risk factors include age less than 1 year 13.6%, Down syndrome 1.4%, craniofacial abnormalities 1.9%, malnutrition 10.7%, serum electrolyte and urea imbalance 10.3%, cardiovascular disease 3.7%, respiratory disease 7.5%, anaemia 8.9%, haemoglobinopathy 3.3% and coagulopathy 1.9%. There were no specific comorbidities that may likely influence the surgical outcome in majority 70.6% of the patients. Adenotonsillectomy was performed on 43.9% patients. Adenoidectomy only was performed on 24.8% patients. The proportion of adenoidectomy alone performed among the age group was 19.2% and 0.5% forages (1 - 5) and (16 - 18) years respectively. **Conclusion**: Adenoid and tonsils surgery are common pediatric otorhinolaryngologist surgical procedures faced with a different form of surgical barriers in low-income countries. These procedures were faced with a various form of risks and the cause of death in this study was cardiopulmonary complications.

Keywords

Adenotonsillectomy, Adenoidectomy, Tonsillectomy, Indication, Techniques, Complications

1. Introduction

Adenotonsillar surgery is surgical removal of adenoid, tonsils or both at same sitting or different sittings [1] [2] [3]. These may be adenoidectomy when only adenoid is removed; tonsillectomy, when only tonsils are removed; adenotonsillectomy, when both adenoid and tonsils are removed. These various forms of adenoid, tonsillar surgery are one of the surgical procedures performed by otorhinolaryngologists, head and neck surgeon all over the world [4] [5]. It is a common otorhinolaryngological surgical procedure in pediatric age group (age younger than 18 years).

The adenoid, tonsil or both are usually surgically removed in patients with the following: obstructive sleep apnoea, nasal obstruction (persistent or recurrent), disruptive snoring, tonsillitis/adenoiditis (recurrent or chronic), unilateral tonsillar enlargement, peritonsillar abscess (quinsy), complications of adenoid and tonsillar disorder such as malnutrition, otitis media, cardiopulmonary disorder, recurrent chest infection, rhinosinusitis, which are resistant to appropriate antibiotic and other medical therapy [6] [7] [8] [9]. Regional indications of adenotonsillectomy may be a component of other procedures such as uvulopalatopharyngoplasty, components in avulsion of symptomatic elongated styloid process [6]. A systemic indication of adenotonsillectomy includes when the tonsils become a focus for distant infection, such as infective endocarditis and glomerulonephritis [6] [10]. The indications for these surgical operations are numerous,

different in the center and often controversial among the otorhinolaryngologist, pediatrician and family physician [11] [12] [13].

There are the various forms of barriers that limit access of the patients to otorhinolaryngological surgery such as adenotonsillectomy in low-income countries. These are further classified into three main types of surgical barriers and they are significantly financial (affordability), cultural (acceptability) and structural (accessibility) surgical barriers [14] [15] [16]. Affordability consists of individuals who had no money for health care, transportation and so on. Accessibility consists of individuals, for whom surgical care was not available to a health facility, personnel or equipment. Acceptability consists of individuals who did not want to undergo surgery due to fear or no trust.

There are different methods of adenoid and tonsillar surgical removal. The traditional, steel blunt dissection technique of tonsillectomy, while the early method of adenoidectomy was by using curette adenoidectomy techniques, is still popular among many otorhinolaryngologists, head and neck surgeons [17] [18] [19] [20]. The other methods are monopolar electrocautery, Electrocauterization, coblation tonsillectomy, Radiofrequency ablation (also called somnoplasty), peak plasma blade, harmonic scalpel tonsillectomy and powered intracapsular tonsillectomy using microdebrider which is some of the most recent techniques of tonsillectomy [17] [18]. Some recent advances in modern techniques of adenoidectomy include microdebridement and functional surgery instruments, electrocautery ablation, coblation, co2 laser adenoidectomy and powered instrumentation [19] [20]. There has been a surgical revolution in modernizing the surgical instruments and techniques of adenotonsillectomy to improve surgical outcome, lower the complications and favorable surgical results. The outcomes of traditional dissection method and curette adenoidectomy techniques are similar to all other forms of adenotonsillectomy surgical techniques [21].

Adenotonsillectomy as the primary treatment adenotonsillar hypertrophy which is the most commonly recognized anatomic risk factor for pediatric obstructive sleep apnoea syndrome is not free of complications. These complications include pain, nausea, vomiting, dehydration, hemorrhage, respiratory decompensation, velopharyngeal incompetence, subglottic stenosis and death [22] [23]. The commonly identified risk factors for postoperative complications in adenotonsillectomy including younger age, obesity, comorbid airway anomalies, Down syndrome and other genetic syndromes, craniofacial abnormalities and neuromuscular disease [24] [25].

There is a paucity of literature on surgical treatment of adenoid and tonsillar disorders in developing countries and Nigeria in particular. This study aimed to determine the rate of adenotonsillectomy, sociodemographic features, indications, barriers to adenotonsillectomy, types of adenotonsillectomy, complications and patients satisfaction post adenotonsillectomy in the developing countries.

2. Materials and Methods

This was a hospital-based retrospective study of pediatric patients who had adenoidectomy, tonsillectomy or adenotonsillectomy. The study was carried out over a period of ten years (from March 2007 to February 2017). This study was carried out in the Ear, Nose, and Throat (ENT) Department of Ekiti State University Teaching Hospital (EKSUTH), Ado Ekiti, Nigeria.

Data for this study was obtained from the medical record department, ENT clinic operation booking register and theatre operation register. The case notes of all the pediatric patients who had adenoid and tonsillar surgery done over the study period were retrieved from the medical record department. Sociodemographic features such as age, gender, parental occupation, parental social habit, domicile and so on were obtained from the patients' case note. Data on patients conditions such as clinical features, diagnosis, indication for surgery, type of adenoid and tonsillar surgery, surgical techniques, complications, and patients satisfaction with the outcome of the surgery were obtained and documented from the case notes.

Those patients that were fit for surgery were properly prepared preoperatively. Those patients that were fit for surgery were then admitted and fasted overnight for at least 6 - 8 the duration of hospital admission were documented. The range of hospitalization varies from 1 - 4 days depending on patients' response to postoperative care. All patients had similar surgical techniques. Adenoid and tonsils did under general anesthesia with orotracheal intubation. The patient was supine positioned and draped. Oral and oropharynx were exposed with the hypopharyngeal pack to protect the lower aerodigestive tract from swallowing and aspiration secretion or blood. Examination under anesthesia was performed pre and postoperatively. Adenoidectomy was performed by curettage of adenoid vegetation. Tonsillectomy was performed by dissection method. Postoperatively feeding on cold diet were encouraged when patients were fully awaked.

Inclusion criteria were all the patients that had adenoid, tonsillar surgery in the department during the study period.

Exclusion criteria were those patients who were not operated. Also patients with incomplete clinical data on this study or those with missing case notes.

Limitation of this study was that instruments for quality of life on adenoid and tonsillar surgery could not be applied. This is a retrospective study.

All the data obtained were collated, documented and statistically analyzed using SPSS version 16. The data were then expressed by descriptive statistics table, bar charts, and pie charts.

Ethical clearance for this study was sought for and obtained from the ethical committee of the institution.

3. Results

A total of 463 patients were booked for adenoid and tonsillar surgery out of which 214 patient had surgery done during the study period. This represented

46.2% of the participants had surgery done.

The age group distribution was shown in Figure 1. The peak was 82 (38.3%) at preschool ages (1 - 5) years while the least age group was 32 (15.0%) at the extreme pediatric ages (16 - 18).

The sociodemographic features of the studied patients were demonstrated in Table 1. There were 126 (58.9%) males and 88 (41.1%) females and the male to

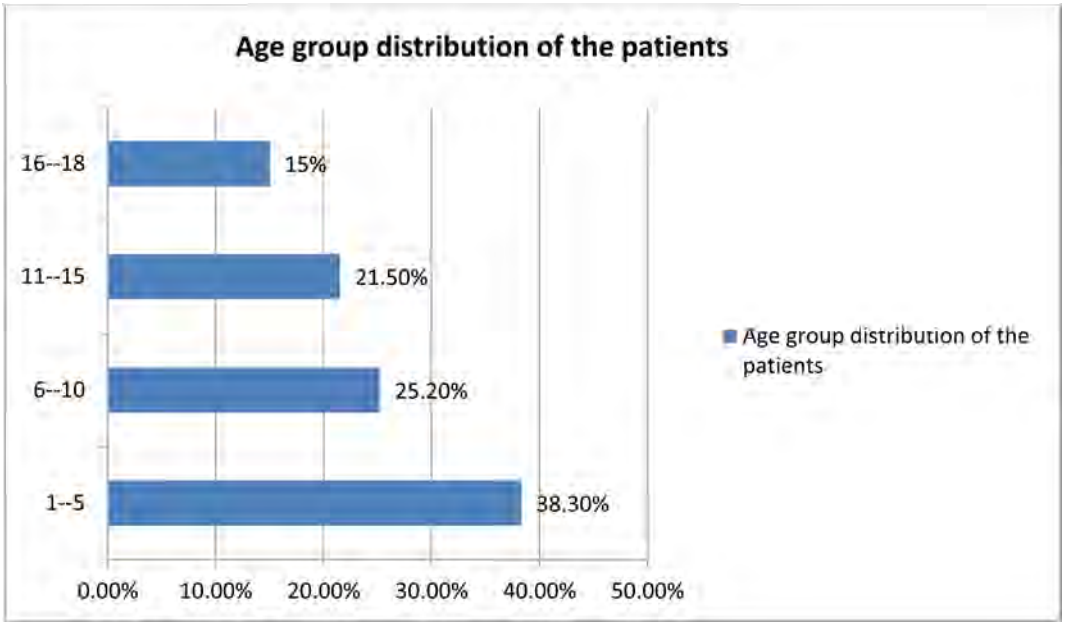

Figure 1. Age group distribution of the patients.

Table 1. Sociodemographic features of the patients.

Sociodemographic features	Number	Percentage (%)
Sex		
Male	126	58.9
Female	88	41.1
Residential		
City	91	42.5
Town	68	31.8
Village	55	25.7
Education level		
Nursery	86	40.2
Primary	53	24.8
Secondary	57	26.6
Post-secondary	18	8.4
Parental occupation		
Healthworker	58	27.1
Business	53	24.8
Teaching	43	20.1
Industrial worker	35	16.4
Farming	25	11.7

female ratio was 1.5:1. Majority 91 (42.5%) of the patients reside in the city while minority 55 (25.7%) of the patients were village dwellers. Nursery or the pre-school ages were the majority 86 (40.2%) of the patients and the minority 18 (8.4%) of the patients were post-secondary schools ages. The parent of the majority of the patients was 58 (27.1%) health workers and 53 (24.8%) businessmen but the parent of the minority of the patients was 25 (11.7%) farmers and 35 (16.4%) industrial workers.

Table 2 illustrated the indications for adenotonsillectomy in the studied patients. Major indications for adenotonsillectomy were 112 (52.3%) obstructive sleep apnoea syndrome secondary to adenotonsillar hypertrophy and 46 (21.5%) recurrent tonsillitis. Less common indications for adenotonsillectomy in this study were 3 (1.4%) persistent fever and 4 (1.9%) cardiopulmonary complications. There were 15 (7.0%) patients admitted as day cases and 199 (93.0%) patients admitted as an inpatient. Postoperatively, 3 (1.4%) of the day cases were admitted as inpatients 4 (1.9%) of inpatients were treated as day cases.

In Figure 2 was an illustration on barriers for adenotonsillectomy. No barriers

Table 2. Indication for adenotonsillectomy.

Indication	Number	Percentage (%)
Obstructive Sleep apnoea	112	52.3
Recurrent tonsillitis	46	21.5
Underweight	16	7.5
Otitis media	14	6.5
Quinsy	13	6.1
Others	6	2.8
Cardiopulmonary complications	4	1.9
Persistent fever	3	1.4

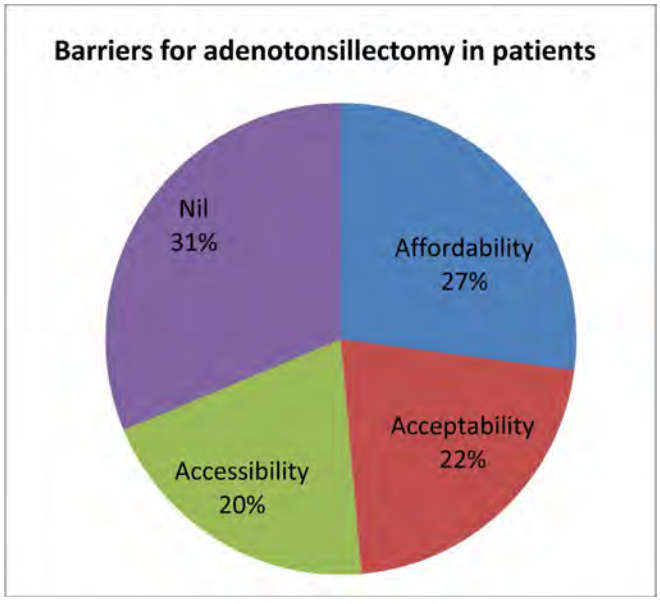

Figure 2. Barriers for adenotonsillectomy in patients.

for adenoid and tonsillar surgery was reported in 67 (31.3%) patients. Barriers such as 58 (27.1%) affordability, 46 (21.5%) acceptability and 43 (20.1%) accessibility were reported by the patients. Affordability in this study includes 24 (11.2%) lack of funds for preoperative investigations and 31 (14.5%) lack of a surgical fee. In acceptability 23 (10.7%) preferred medical treatment to surgery and 21 (9.8%) fear of anesthesia. In this work accessibility was mainly 27 (12.6%) distance patients traveled to the center.

Comorbid illnesses that may affect surgical outcome in patients were illustrated in Table 3. In this study the established high risk factors includes age less than 1 year 29 (13.6%), Down syndrome 3 (1.4%), craniofacial abnormalities 4 (1.9%), malnutrition 23 (10.7%), serum electrolyte and urea imbalance 22 (10.3%), cardiovascular disease 8 (3.7%), respiratory disease 16 (7.5%), anaemia 19 (8.9%), haemoglobinopathy 7 (3.3%) and coagulopathy 4 (1.9%). There were no specific comorbidities that may likely influence the surgical outcome in the majority of 151 (70.6%) of the patients.

Types of adenotonsillectomy among our studied patients were shown in Figure 3.

Table 3. Comorbid illnesses that may affect the surgical outcome in patients.

Comorbid illnesses	Number	Percentage (%)
Ageless than 1 year	29	13.6
Malnutrition	23	10.7
Serumelectrolyteandureaimbalance	22	10.3
Anemia	19	8.9
Respiratory disease	16	7.5
Cardiovascular disease	8	3.7
Haemoglobinopathy	7	3.3
Coagulopathy	4	1.9
Craniofacial abnormalities	4	1.9
Down syndrome	3	1.4

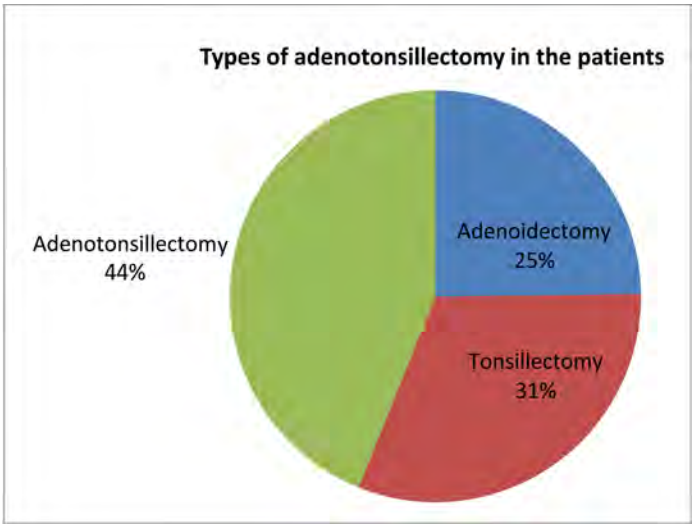

Figure 3. Types of adenotonsillectomy in the patients.

Adenotonsillectomy was performed on 94 (43.9%) patients. Adenoidectomy only was performed on 53 (24.8%) patients. The proportion of adenoidectomy alone among the age group were 41(19.2%), 8(3.7%), 3(1.4%) and 1(0.5%) for ages (1-5), (6-10), (11-15) and (16-18) years respectively. Tonsillectomy only was performed on 67 (31.3%) patients. The proportion of tonsillectomy alone among the age group was nil, 8 (3.7%), 19 (8.9%) and 40 (18.7%) for ages (1 - 5), (6 - 10), (11 - 15) and (16 - 18) years respectively.

Complications associated with adenotonsillectomy among the patients in this study were illustrated in Table 4. No complications were recorded in 12 (5.6%) patients. Most common complications of adenoid and tonsillar surgery in this study were 196 (91.6%) pain at surgical site/odynophagia and 103 (48.1%) vomiting. Less common complications of adenoid and tonsillar surgery in this study were 4 (1.9%) cardiac arrest, 14 (6.5%) otalgia and 18 (8.4%) soft tissue injury. There were 2 (0.9%) death recorded from cardiac arrest {1 (0.5%) preoperative and 1 (0.5%) postoperative}.

Demonstrated in Figure 4 was patients' satisfaction with adenoid and tonsillar

Table 4. Complications associated with adenotonsillectomy in the patients.

Complications	Number	Percentage (%)
Pain surgical site/odynophagia	196	91.6
Vomiting	103	48.1
Nasal regurgitation	23	10.7
Soft tissue injury	18	8.4
Otalgia	14	6.5
Nilcomplication	12	5.6
Cardiac arrest	4	1.9

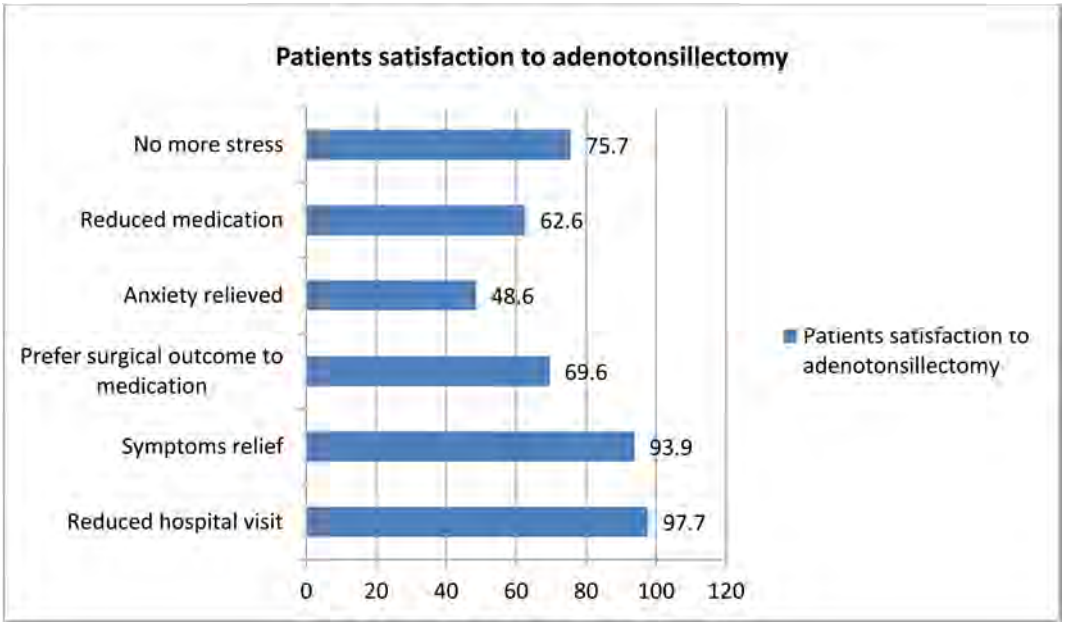

Figure 4. Patients satisfaction to adenotonsillectomy.

surgery postoperatively. Majority of the patients were satisfied with the surgery as 209 (97.7%) reduced hospital visit, 201 (93.9%) symptoms relief and 162 (75.7%) no more stress. The minority of the patients were satisfied with the surgery as 104 (48.6%) anxiety relief and 134 (62.6%) reduced medication.

4. Discussion

Adenoid and tonsillar surgery are common pediatric otorhinolaryngological head and neck surgery worldwide in the ear, nose and throat practice. In this study, the proportion of booked patients for the surgery was about half of the population. These were due to one or more barriers to surgery among the patients. The most significant barriers to adenotonsillectomy surgical uptake by the patients were financial barriers. These include the inability to pay for costs of medication, preoperative investigations, admission fee, surgical fee and so on. Most of these patients are a pediatric age group, dependant and they were sponsored by parents, guardian, relatives, and nonrelative. Accessibility to the center is another major barriers. Majority of the patients resided outside the central location or from neighboring state. Most of the patients preferred alternative treatment to adenotonsillectomy. This is not only because of the surgical fee but patients fear surgery, fear anesthesia, bleeding, pain at the surgical site and so on. All these barriers to surgery and other intervention were documented in previous studies [14] [15] [16] [26].

Preschool age, (1 - 5) years group were predominant in this study more than another age group this may be due to a higher incidence of upper respiratory tract infection and higher activities of lymphoid tissue at the entrance of upper aerodigestive tract. These findings are similar to another study on the pathology of pediatric tonsils [27].

In this study, male patients were commonly scheduled for adenoid and tonsils surgery than the female counterparts. This gender disparity may be as a result of parents in developing and low-income country care more for male children than female children. Male children generally play rough and they are prone to traumatic injury and infection. These findings are similar to report from other study [21].

Adenoid and tonsils surgery in this study were commonly indicated in patients with obstructive adenotonsillar hypertrophy and recurrent tonsillitis. Obstructive adenotonsillar hypertrophy leads to blockage of nasopharyngeal airflow, mouth breathing, snoring and finally obstructive sleep apnoea syndrome. Adenotonsillar hypertrophy may be secondary to allergy or microbes. Mucous discharge stasis from obstructive adenotonsillar hypertrophy is a good culture medium for microbes. Superimposed bacterial infection leads to infection such as recurrent tonsillitis, quinsy, otitis media, and so on. A further effect of adenotonsillar hypertrophy was a loss of olfaction with resultant loss of appetite and malnutrition. These findings were reported in other studies [28] [29].

When adenoid and tonsils surgery was indicated in this study only very few

patients did not experience any form of barriers before the surgery was performed. Majority of the patients experience barriers before the surgery was performed. Commonest barriers were from financing the various aspects of the surgery. This is most likely due to a minority of patients resides in the city, the majority were dependants and the majority of the parents were salary earners (teachers, industrial workers, and health workers). The financial obligations include the cost of laboratory investigations, management, and stabilization for the associated comorbid illnesses in these patients. The main components of the comorbid illnesses include pediatric less than one year, malnutrition, serum electrolyte, and urea imbalance, anemia and cardiopulmonary disorder in the patients.

In this study in the low-income country, tonsillectomy was performed by cold steel dissection method while adenoidectomy was performed by adenoid curette. The commonly performed adenoid and tonsils surgery in this study was adenotonsillectomy while the least surgery was the only adenoidectomy. Adenoidectomy and adenotonsillectomy in this study were mainly secondary to obstructive adenotonsillar hypertrophy with obstructive symptoms such as obstructive sleep apnoea syndrome. This is commoner in much younger paediatric. Similar to this study, adenoidectomy and adenotonsillectomy exclusively indicated in pediatric age group with adenotonsillar hypertrophy in other study [30]. Main indication for tonsillectomy alone in this studied patient was the infection of the lymphoid tissue. The commonly encountered infection in this study was recurrent tonsillitis, peritonsillar abscess (quinsy). These conditions were commoner older pediatric. These findings agree with reports from another study where tonsils infections were the most common indication for tonsillectomy in young adult age group [31].

Common complications encountered in this study of the adenotonsillectomy, adenoidectomy and tonsillectomy were a pain at the surgical site with associated odynophagia. In older pediatric age group there was associated referred otalgia. This is the most common complications among the patients and it is managed immediately postoperatively with intravenous pentazocine and paracetamol. Similarly, drugs were used to manage postoperative pain in adenotonsillar surgery in another study (Strauss, 2012). Maximum pain toleration occurs within 24 hours (first day) post-operative period to the enabled the majority of patients to tolerate cold liquid diet. Ice cream or cold fluid diet was recommended for the patients from when they were fully awake/recover from anesthesia. This practice reduced the proportion of postoperative dehydration among the studied patients. The next most common complication in this study was vomiting. The vomitous in few patients contains alter chocolate brown clotted blood. The vomiting was mainly once. The oropharyngeal examination was done with normal findings. There was no recorded postoperative hemorrhage and surgical infection in all the patients this may be because all the patients were operated by consultant otorhinolaryngologist. The center has not been accredited for residents training. All the patients had prophylaxis antibiotic, intravenous Augmen-

tin (clavunated Amoxicillin). The most deadly complication recorded in this study was perioperative cardiac arrest which is 50% fatal. Two death was recorded out of four patients. One was during induction anesthesia and the second was during extubation of the patients.

About 75% of the patients were fit for discharge on the first day (24 hours) postoperatively. Few (5%) were treated as day cases based on their clinical state and majority had adenoidectomy alone.

The indications for the adenoid and tonsils surgeries were completely resolved in the pediatric in this study. Parents were satisfied with surgery compared to prolonged medical and conservative therapy. Apart from symptoms relief parents also enjoyed reduced hospital visit, reduced medication, and stress with anxiety was no more. These findings are similar to report from other studies[3].

5. Conclusion

Adenoid and tonsils surgery predominant indicated in pediatrics with the adenotonsillar disorder with failed medical and conservative therapy worldwide. Similarly, adenotonsillar surgery was faced with a various form of surgical barriers and risk factors. In a low-income country, the surgical barriers and risk factors must be borne in mind while preparing such patients for surgery to reduce morbidity and mortality.

Limitation of the Study

The limitation of the study is that it is hospital-based and the data can not represent the entire country.

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Conflicts of Interest

All the authors declare that there were no competing interests.

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