

BACTERIAL AND FUNGAL AGENTS OF DIARRHOEA AMONG UNDER-FIVES IN TWO TERTIARY HOSPITALS IN SOUTHERN NIGERIA**Dirisu John O.*¹, Agbakoba Nneka R.², Okwelogu Somadina I.³, Eki-Udoko Fidelis E.⁴, Elo-Ilo Jacinta C.⁵ & Oladipo Olukayode⁶**¹Department of Virology, Infection Sciences, Severn Pathology, UK Health Security Agency, Bristol, England & Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.^{2,3}Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.⁴Department of Paediatrics, University of Benin Teaching Hospital, Benin City, Nigeria.⁵Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria.⁶Institute of Child Health, University of Benin Teaching Hospital, Benin City, Nigeria.***Corresponding Author: Dr. Dirisu John O.**

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ABSTRACT

Diarrhoeal disease is a leading cause of morbidity and mortality globally in children under five years of age, especially in developing countries like Nigeria. Although a lot of studies have been done on unraveling the causative agents of bacterial and parasitic diarrhoea in under-fives across the world including Nigeria, only a limited number of these studies have attempted to consider the synergistic effect of bacterial and fungal pathogens on diarrhoea in children who are under five years of age in our locality. It is against this background that this study was done. This was a cross-sectional study carried out at the University of Benin Teaching Hospital (UBTH) and Nnamdi Azikiwe University Teaching Hospital (NAUTH) in Benin City and Nnewi respectively. Three hundred and thirty participants were recruited for the study using stratified random sampling, aged between 1 and 5 years. Stool samples were obtained and cultured using standard techniques, while the bacterial and fungal pathogens were isolated after relevant biochemical tests had been carried out. Data from the study were analyzed using chi-square and level of significance expressed using p-value ($p < 0.05$). Three bacterial isolates namely, *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* were isolated from the stool samples assayed for this study with the most being *Escherichia coli* (52.9%; 45/85). *Candida albicans* (62.2%; 23/37), *Candida tropicalis* (24.3%; 9/37) and *Candida glabrata* (13.5%; 5/37) were the predominant fungi. However, age and sex had no statistically significant effect on bacterial and fungal diarrhoea in the under-fives respectively ($p > 0.05$; $\chi^2 = 21.06$).

KEYWORDS: Diarrhoea, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*.**INTRODUCTION**

Diarrhoea is defined as having loose or watery stools at least three times per day, or more frequently than normal for an individual.^[1] While most episodes of childhood diarrhoea are mild, acute cases can lead to significant fluid loss and dehydration, which may result in death or other severe consequences if fluids are not replaced at the first sign of diarrhea.^[1] Although the burden of diarrheal disease worldwide is high, developing countries such as Nigeria account for over two million deaths of young children per year.^[2] Although a lot of studies have been done on unraveling the causative agents of bacterial and parasitic diarrhoea in under-fives across the world including Nigeria,^[3] only a limited number of these studies have attempted to consider the synergistic effect of bacterial and fungal pathogens on diarrhoea in

children who are under five years of age in our locality. This study was aimed at identifying the bacterial and fungal agents responsible for childhood diarrhoea in under-fives.

MATERIALS AND METHODS

A total of 330 diarrhoeic stool samples were collected aseptically using sterile wide mouth containers from the children in the wards when they defaecate. These samples were processed using microscopy, culture and sensitivity within 6 hours of collection.

Microscopy, culture and sensitivity

One hundred (100) of the 330 stool samples collected were selected based on duration and severity of diarrhoea and cultured using aseptic techniques on Salmonella-

Shigella agar, Mackonkey and Sabouraud dextrose agar for 48 hours at 37°C to check for the presence of both bacteria and fungi isolates. Briefly, culture was done by flaming the wire loop until it became red hot, allowed to cool down and a small portion of the stool sample picked and emulsified on the agar to make a well of inoculum. Thereafter, the wire loop was flamed again, allowed to cool and the primary, secondary and tertiary streaks were done while flaming intermittently to obtain discrete colonies and avoid contamination. The plates were thereafter incubated in an incubator at 37°C for 48 hours to observe bacterial and fungal growth colonies. This was done using normal saline and iodine as described previously by.^[4]

Biochemical tests

After 48 hours of incubation, the agar plates were observed macroscopically for bacterial and fungal growth: to enable the identification of the growths seen, Gram staining and necessary biochemical tests were done including indole test, citrate utilization test, urease test and motility test.^[5]

Presumptive identification of fungal isolates

Using already prepared CHROMagar Candida™ plates, the Candida isolates were streaked carefully and incubated for 48 hours before they were read according to the manufacturer's instruction.^[6]

RESULTS

Table i: Prevalence of bacterial isolates by percentage with respect to age and sex in diarrhoeic stool samples of under-fives in NAUTH, Nnewi and UBTH, Benin City.

Isolate	No. Isolated	% prevalence	Age (months)			Sex	
			1-20	21-40	41-60	Male	Female
<i>K. pneumoniae</i>	31.0	36.5	7.0	15.0	6.0	19.0	12.0
<i>E. coli</i>	45.0	52.9	10.0	27.0	8.0	17.0	28.0
<i>P. mirabilis</i>	9.0	10.5	2.0	7.0	1.0	3.0	6.0
Total	85		19.0	49.0	15.0	39.0	46.0

$\chi^2 = 21.06, p > 0.05$

K. pneumoniae = *Klebsiella pneumoniae*, *E. coli* = *Escherichia coli*, *P. mirabilis* = *Proteus mirabilis*,

Table ii: Prevalence of fungal isolates by percentage with respect to age and sex in diarrhoeic stool samples of under-fives in NAUTH, Nnewi and UBTH, Benin City.

Isolate	No. Isolated	% prevalence	Age (months)			Sex	
			1-20	21-40	41-60	Male	Female
<i>C. albicans</i>	23	62.2	8.0	12.0	3.0	9.0	14.0
<i>C. tropicalis</i>	9.0	24.3	3.0	5.0	1.0	3.0	6.0
<i>C. glabrata</i>	5.0	13.5	2.0	3.0	0	1.0	4.0
Total	37		13	20.0	4.0	13.0	24.0

$\chi^2 = 21.06, p > 0.05$

C. albicans = *Candida albicans*, *C. tropicalis* = *Candida tropicalis*, *C. glabrata* = *Candida glabrata*

Three bacterial and fungal species were isolated from the stool samples are shown in Table (i) above, with the age group 21-40 months most affected by the diarrhoea. More females had diarrhoea than their males counterparts, but age and sex did not have any statistically significant effect on diarrhoea (0.05; $\chi^2 = 21.06$). Among the bacterial isolates, *Escherichia coli* was the most dominant, responsible for 52.9% of the diarrhoeic cases (Table i). While *Candida albicans* was the most prevalent fungal isolate among the under-fives used from both study locations (62.2%) (Table ii). The demographic distribution of the under-fives in both study locations are shown in figures i and ii below.

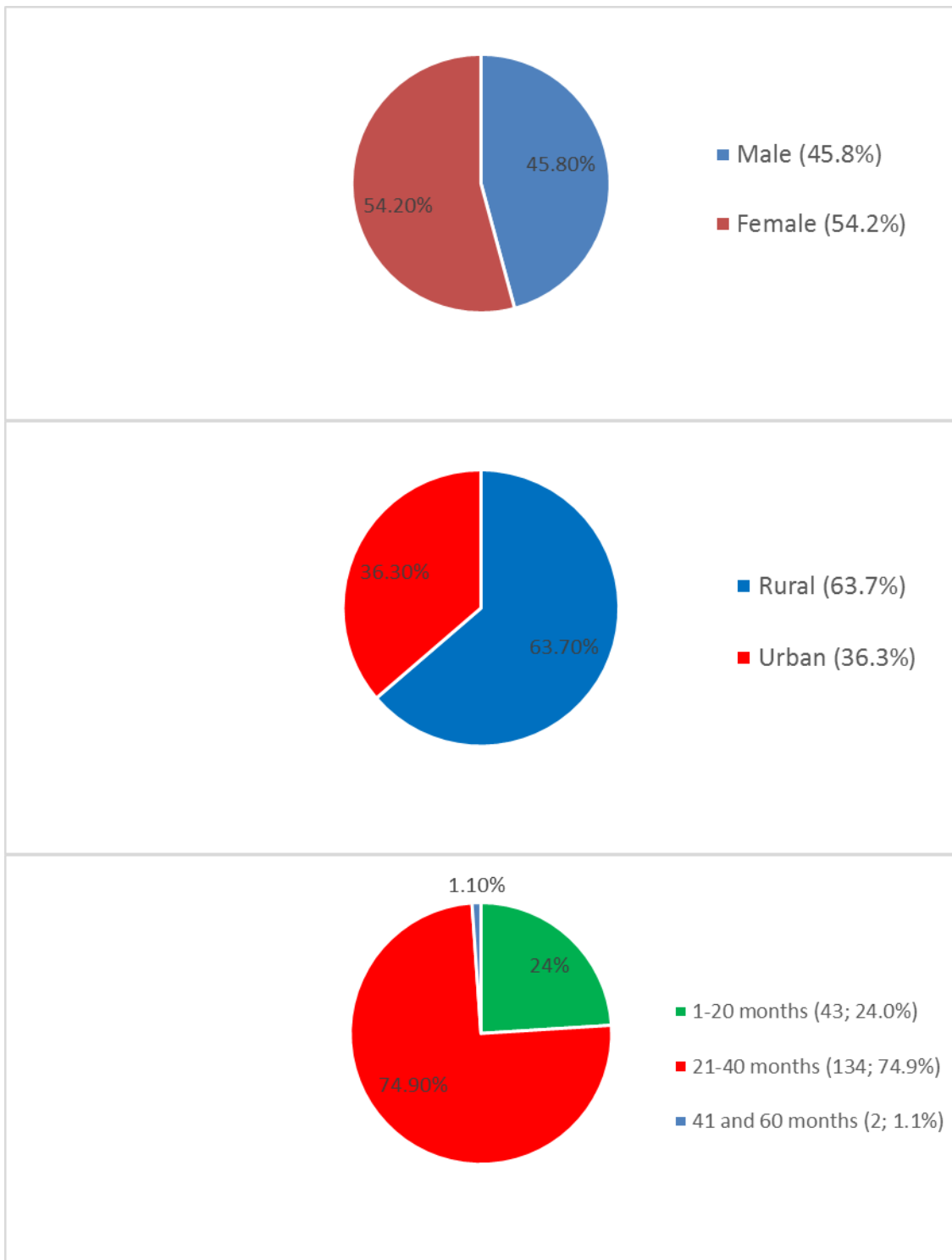


Figure i: Demographic information of under-fives attending the Nnamdi Azikiwe University Teaching Hospital, Nnewi.

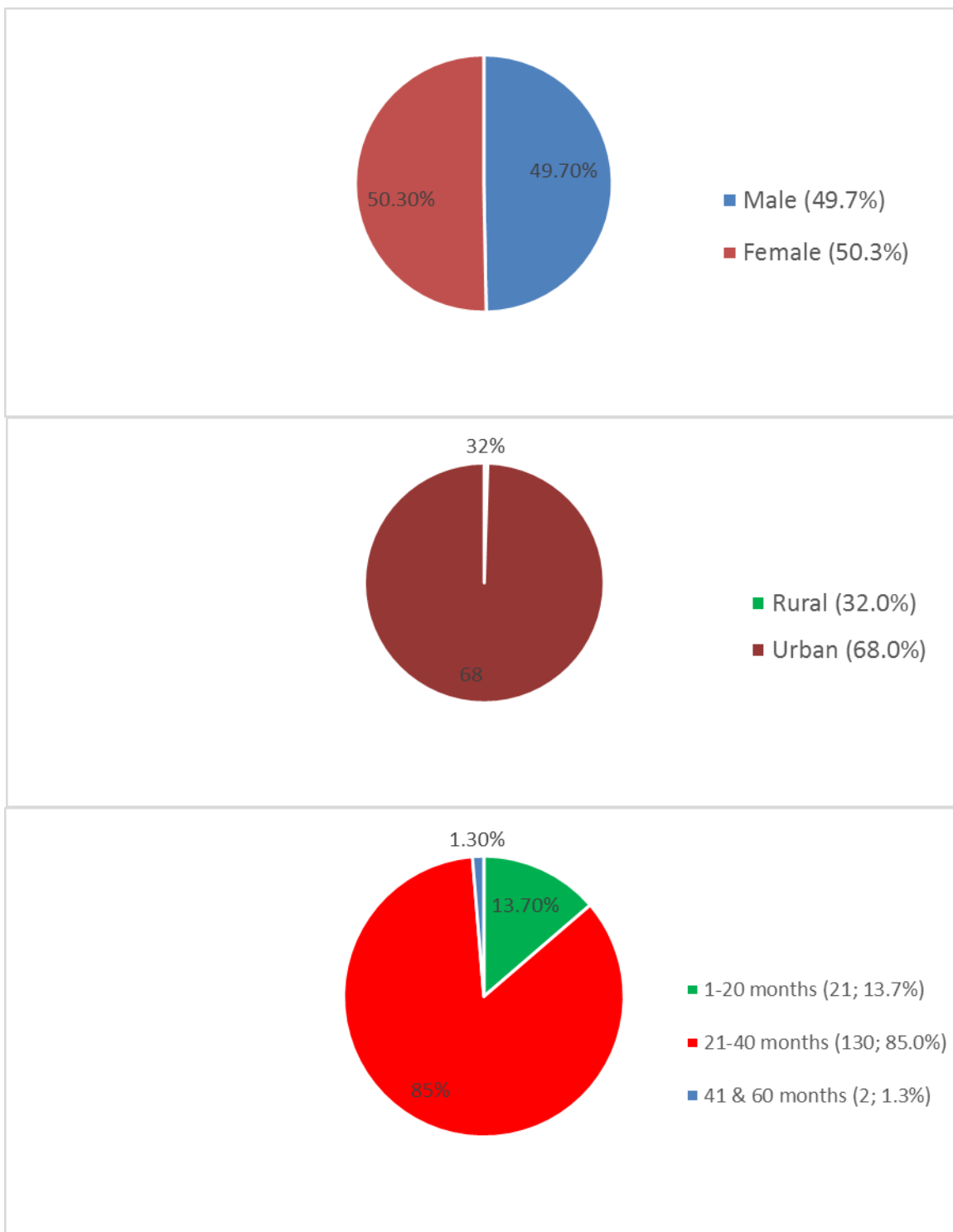


Figure ii: Demographic information of under-fives attending the University of Benin Teaching Hospital, Benin City.

DISCUSSION

In this study, three bacterial agents of diarrhoea were isolated namely *Esherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* with prevalence of 52.9%, 36.5%, 10.5% respectively. This is similar to an earlier report by^[7] from the children at a daycare recruited for their study and a recent finding from Tanzania.^[8] The implication of this finding is that the presence of these

bacterial agents occurring at the same time makes the diarrhoea more persistent despite initial medical intervention, such as antibiotic therapies.

It is known that bacterial agents constitute a major portion of the gastrointestinal tract and when adequate safety precautions are not taken, they could find their way through the faecal-oral route and cause infections. This may have been the reason for the high burden of

bacterial agents of diarrhoea observed in this study, especially in the males between the ages of 21-40 months. A recent study done in South Africa also lends credence to the results from this research.^[9]

Fungal agents such as *Candida* have also been implicated in diarrhoea as have been reported earlier.^[10,11] The species of *Candida* isolated from this study were *Candida albicans* (62.2%), *Candida tropicalis* (24.3%) and *Candida glabrata* (13.5%) similar to those reported by.^[11,12]

The contribution of fungal agents to diarrhoea may not have been well elucidated as available studies suggest in some quarters that fungi agents can cause diarrhoea while others suggest otherwise.^[11] Although, this argument exists, the finding of three species of *Candida* in this study (*Candida albicans*, *Candida tropicalis* and *Candida glabrata*) suggests that *Candida* may be associated with diarrhoea in children who are under five years of age, but presenting with bacterial diarrhoeic symptoms which can mislead diagnostic steps aimed at nipping it in the bud. This may result in the child having longer stay at the hospital with its attendant economic burden on the parents and society. It has however been reported that the overuse of antibiotics by children, especially in Nigeria usually causes *Candida* overgrowth,^[6] which could have accounted for the figures obtained in this study for *Candida* related diarrhoea.

While in many scenario the agent of an infection may be a particular causative agent, this study showed that there is co-infection between the agents of bacterial and fungal diarrhoea, as has been previously reported.^[13] This study concluded that age and sex had no statistically significant relationship with bacterial and fungal diarrhoea among under-fives. However, there is a need to monitor diarrhoea in under-fives with a bid to establishing the exact causative agent, so as to put the most effective control measures in place.

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Conflict of interest

The authors declare that there is no conflict of interest.

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