Isolated and Identification of some pathogenic bacteria in children with diarrhea and respiratory tract infection

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Abstract :

The main objectives of the present study were to determine the isolation of main pathogenic bacteria that cause diarrhea and respiratory tract infection in children under 5 years of ageby using specific parameters.

Bacteriological analysis showed that from 50 stool specimens ; 21(42%) were strains of *Escherichia coli* others are *Klebsiella pneumonia* 9(18%) , *Enterobacter cloacae* 8(16%) and other Enterobacteriacea , and the distribution of diarrheal children by age . It shows that diarrhea is statistically associated with age and majority of cases occurring in children between 7months and under 5 years of age.

Bacteria were isolated from 15 of 47 (31.91%) nasal swabs *Staphylococcus aureus* was most common , isolated in 10 out of the 47 (21.3%) nasal swabs collected from the children followed by *E. coli* , 9 (19.14%) β-hemolytic *Streptococcus* , 6 (12.3%) *Klebsiella pneumonia* . Other bacteria isolated in fewer specimens were *Enterococcus faecalis* (10.63%) and *Enterobacter spp.*(4.25%) .

In this study, bacteremia was confirmed in 28(56%) patient out of 50 children. the most common primary infection was identified in 50(33.3%) from 150 samples: gastroenteritis and pneumonia 47(31.3%) .The most common pathogens were *Streptococcus spp.* (35.7%) especially Beta hemolysis Streptococci mainly isolated from cases with pneumonia.

Escherichia coli was isolated in (17.9%) of the cases ; it was reported to be the most frequent gram-negative bacterial species recovered from blood cultures , *Klebsiella pneumonia* presented (14.3%) of the cases , *Enterococcus faecalis* and *Enterobacterspp.*

Were isolated from blood cultures of three children (7.14% , 3.6%) who had been hospitalized for > 10 days.

Introduction:

Diarrhea disease is one of the leading causes of illness in young children in developing countries (1) . Diarrhea diseases are the cause of almost three million deaths annually mainly among children younger than five years of age (2) . Although extensive investigation of diarrhea have not been reported.The diarrhea – specific mortality in children younger than five years of age in Africa has been estimated at about 106 per 1000.Infectious diarrhea is a major cause of morbidity and mortality in infants and young children worldwide (1). Infectious diarrhea can be classified into two groups, invasive, when fecal leukocytes are present in stool and noninvasive, when fecal leukocytes are absent or only scattered in stool (2).

Diverse studies have demonstrated that malnutrition increases the risks of infection and death (3). The most frequent causes of death in children under 5 years old are acute diarrhea and acute respiratory infection. Several studies have shown that malnutrition is frequently causally associated with these deaths (4). However, as malnutrition rarely appears as cause of death on death certificates, its impact is largely underestimated.

Children are at risk of gastrointestinal infections because of their physiological and immunological status. Their immune system is still immature; the B cells are not as well developed and do not react to antigenic stimuli as effectively. The IgA levels, which are important against microbial infections, increase with age. The levels of this immunoglobulin in adults are 70-300 times that of newborn. In children, the digestive system is not fully developed and sometimes it cannot act as a barrier that prevents infections (5).

The main aetiology of the diarrhoea is related to a wide range of bacteria, enteroparasites and viruses (6). The contribution of the various pathogens to diarrhoea may differ substantially between regions depending on local meteorological, geographic, and socio-economic conditions (7). Underlying reasons for the spread of diarrhoeal diseases are found in poor hygiene and sanitation, limited access to safe drinking water as well as in inadequate education of health care providers and recipients (8).

Bacteremia in certain infection is considered the best practical way to identify the causative organism. The isolation of bacteria from blood cultures is usually indicative of a serious invasive infection that requires immediate antibiotic treatment (9). Septicemia is a pathological condition with a high mortality rate that varies between 30 and 70 per cent and depends on several factors including virulence of the pathogen and host factors .The majority of the bacteremia cases are caused by a number of pathogens including *Staphylococcus spp.*, S*treptococcus*

spp.,Enterobacter spp.,Escherichia coli,Klebsiella pneumonia and *Pseudomonas spp*. (10, 11).

The followings aspects were considered during the study period:

1- Isolation of main pathogenic bacteria that causing diarrhea and Respiratory tract infection in children under 5 years old.

2- Determination of bacterial pathogenicity in diarrhea and its relationship with age.

Materials& Methods

1-Materials

1-1 Culture Media

All the following media were prepared according to company product and sterilized by autoclaved at 121 C°, and 15 pound/inch^2 for 15min.

1- MacConkey Agar

Used for isolation and differentiation between bacteria which ferment and non-ferment lactose for Enterobacteraeceisolation.

2 -Eosin methylene blue Agar (EMB)

A differential medium for the isolation of coliform organisms, as selective and differential media for Klebsiella and *E.coli* to produce metallic sheen on culture.

3-Brain –heart infusion broth

Used for activating, growing and as stock culture for bacterial isolates .

4-Simmon – citrate medium

Used for examing the ability of bacteria to utilize of sodium citrate as the carbonsource, autoclaved and poured the media as slant in sterile tubes.

5 – Triple sugar Iron (TSI)

Differential medium for isolation of gram negative enteric bacilli from clinical .

6 - Methyl red – Vogesproskauremedium:

Used for detecting the ability of bacteria to ferment glucose and producing acid as well as acetone, prepared according (12) as follow :

Peptone 5gm
K2HPO4 5gm
Distilled water 950 ml

pH was adjusted to 7.6, sterilized by autoclave ,then media was cooled and added 50 ml of 10% glucose solution which filtrated by Millipore filter 0.22 Mm ,then divided as 5ml in sterile tubes

2- Methods:

2-1 Study design:

Descriptive case study was carried out to determine common bacterial aetiology of diarrheal diseases and respiratory tract infection in the children under 5 years old of age. Fifty children under than five years of age who had gastroenteritis (diarrhea) and respiratory tract infection both out patients and those who were admitted were selected from Al SaderTeaching Hospital were presented to pediatrician.

2-2 Method of data collection:

Most of the patients were admitted to the hospital with symptoms of fever, vomiting, diarrhoea and dehydration. The relevant clinical information was collected by filling the questionnaire for each patient. The requested data included age, sex, duration of diarrhoea and history of antibiotic therapy prior to the clinic visit.

2-3 Microbiology study:

A single faecal sample and swabs were collected from each child with diarrhoea and upper respiratory infection on presenting at the hospital . The relevant clinical data were recorded in a preform. Faeces of children with the presence of blood and mucous were excluded from the study. The faecal samples and swabs were cultured on differential and selective media for bacteria cultivation in order to isolate bacteria

A blood specimen was collected from each patient and inoculated in broth media (Nutrient broth) were incubated at 37C° and continuously monitored for evidence of bacteria growth. An aliquot of the positive blood culture was taken by a syringe for Gram stain and subculture on enriched and selective media for a wide variety of pathogens .

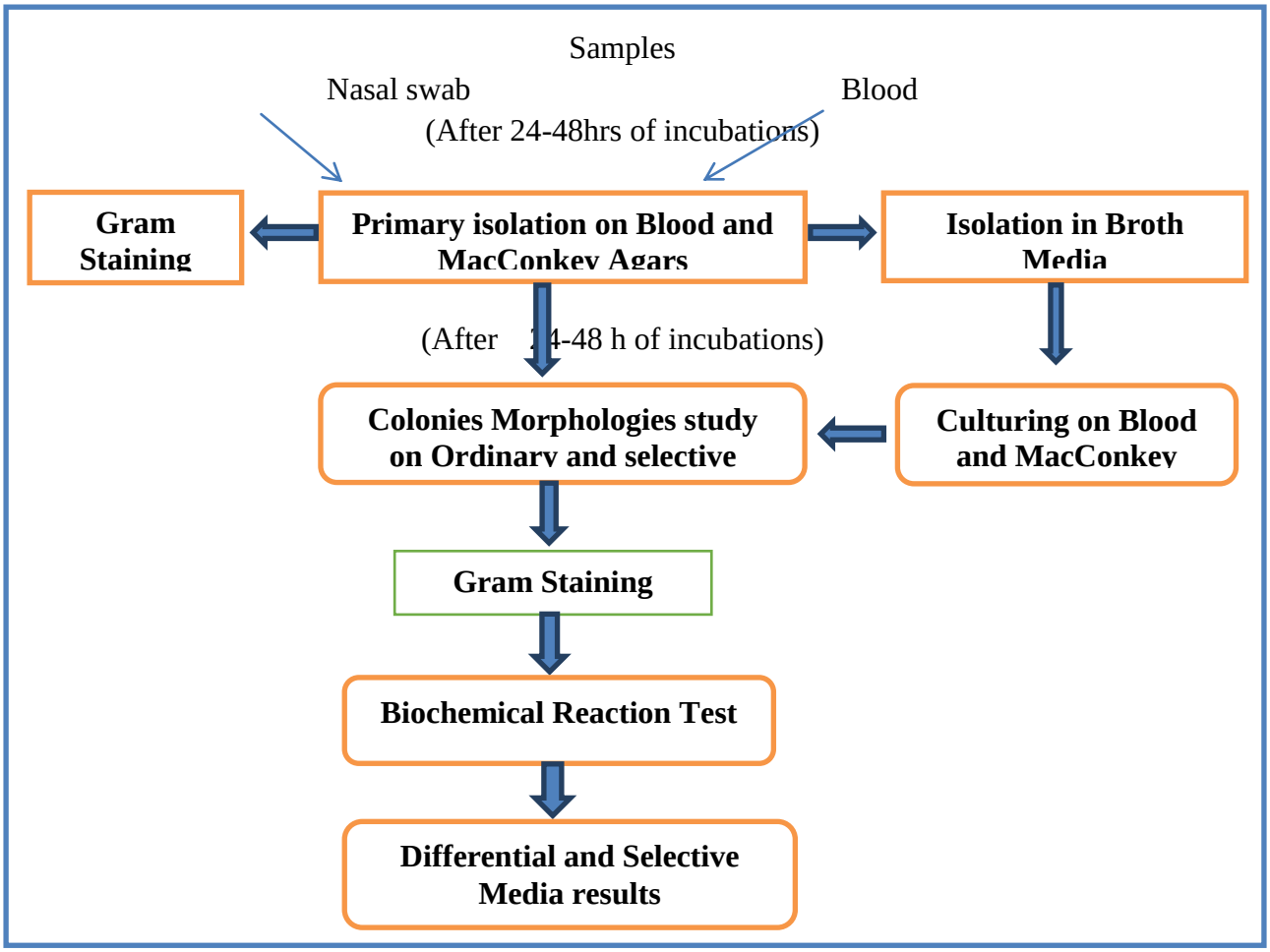

Figure 3 : The primary isolation steps of bacterial from nasal and swabs samples (13) .

2-4 Statistical analysis :

Data were given as ANOVA test were used to find out the significant differences. The statistical significance was accepted as $P< 0.05$, 0.01. ,and data analysis by using SPSS ,(14) .

4- Results

Fifty children under five years old of age with acute diarrhoea and respiratory tract infection were included in the study.

Bacteriological analysis showed that bacterial isolate from 50 stool specimens ; 21(42%) were strains of *Escherichia coli* others are *Klebsiella pneumonia* 9(18%), *Enterobacter cloacae* 8(16%) and other Enterobacteriacea. Data are shown (Table 1).

Table (2) shows the distribution of children with diarrhoea by age. It shows that diarrhoea is statistically associated with age and majority of cases occurring in children between 7months and under 5 years of age .

Bacteria were isolated from 15 of 47 (31.91%) nasal swabs *Staphylococcus aureus* was most common , isolated in 10 out of the 47 (21.3%) nasal swabs collected from the children followed by *E. coli*, 9 (19.14%) β-hemolytic *Streptococcus* , 6 (12.3%) *Klebsiella pneumonia*. Other bacteria isolated in fewer specimens were *Enterococcus faecalis* (10.63%) and *Enterobacter spp.*(4.25%) .

In this study, bacteremia was confirmed in 28(56%) patient out of 50 children . the most common primary infection was identified in 50(33.3%) from 150 samples :gastroenteritis and pneumonia 47(31.3%). The most common pathogens were *Streptococcus spp.* (35.7%) especially Beta hemolysis Streptococci mainly isolated from cases with pneumonia . *Escherichia coli* was isolated in (17.9%) of the cases ; it was reported to be the most frequent gram-negative bacterial species recovered from blood cultures . *Klebsiella pneumonia* presented (14.3%) of the cases. *Enterococcus faecalis* and *Enterobacterspp.* were isolated from blood cultures of three children (7.14% , 3.6%) who had been hospitalized for > 10 days. No pathogenic bacteria were isolated from other sample (Table 3) .

Table 1: Distribution of Bacterial Organisms Isolated from children with diarrhoea.

Bacterial isolate	Total number of Bacteria Isolated	percentage(%) of samples analysis
Escherichia coli	21	42%
Klebsiella pneumonia	9	18%
Enterobacter cloacae	8	16%
Proteus species	5	10%
Enterococcus faecalis	4	8%
Salmonella species	3	6%
Total and percentage	50	100%

Table 2: Distribution of isolated pathogenic bacteria according to age.

Bacterial isolate	Less than 1 year	1—2 years	2—5 Years	Total
Escherichia coli	10 (76.9%)	8(38%)	3(18.75%)	21
Klebsiella pneumonia	1(7.6%)	5(23.8%)	3(18.75%)	9
Enterobacter cloacae	1(7.6%)	6(28.57%)	1(6.25%)	8
Proteus species	1(7.6%)	1(4.7%)	3(18.75%)	5
Enterococcus faecalis	0(0%)	1(4.7%)	3(18.75%)	4
Salmonella species	0(0%)	0(0%)	3(18.75%)	3
Total and percentage	13	21	16	50

Table 3: Distribution of Bacterial Organisms Isolated from Swab and Blood sample of children.

Bacterial isolate	Total number of Bacteria Isolated and percentage (%) of samples analysis			
	Swab		Blood sample	
Escherichia coli	10	21.3 %	5	17.9 %
Klebsiella pneumonia	6	12.76 %	4	14.3%
Enterobacter spp.	2	4.25%	1	3.6%
β-hemolytic *Streptococcus*	9	19.14 %	10	35.7%
Enterococcus faecalis	5	10.63 %	2	7.14%
Staphylococcus aureus	15	31.91 %	6	21.4%
Total and percentage	47	100	28	100

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Discussion

Acute diarrhoea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Nigeria (15). Clarification of the enteropathogens involved in diarrhoeal disease is an essential step towards the implementation of effective primary health care activities against the disease (16). In this study our result shows that sixth bacterial species (*Escherichia coli, Klebsiella pneumonia, Enterobacter spp. Enterococcus cloacae , Proteus species* and *Salmonella species*)were isolated from children withdiarrhoea.

This study determined the significance and the association of the strains of *Escherichia coli* as a predominant enteropathogen causing diarrhea in children younger than 5 years in Abuja, Nigeria (17). We shall subsequently seek to further clarify this association with the respective diarrhoegenic*E.coli* categories by investigating their virulence properties . The prevalence of cases of diarrhoea in Abuja, Nigeria with a potential bacterial pathogen detected was 65.8% of all patients screened. This though in contrast to a recent report of 83.1% from similar study in Abakaliki, south –eastern Nigeria (18,19) is consistent with the reports of 63.3%-71.83% in Tanzania and 50-60% in other developing countries (6). The variation in prevalence between the two Nigerian cities might be attributed to differences in infrastructural and socioeconomic indices. The percentage of diarrhoea cases from which bacteria was isolated ranged from 18.8% to 22.4% among stool samples from centers located in the Municipality and 18.1% to 20.9% from the centers in the satellite settlements. (2).

Escherichia coli and *Klebsiella*species were isolated at a relatively high rate. Statistical analysis showed that *Escherichia coli was* significantly associated with diarrhoea in children younger than 2 years ($P<0.05$). There appear to be conflicting reports about the association of *Salmonella* species with diarrhoea (2). Conversely, the occurrence of *Salmonella* species in this study is in conformity with the findings from Abakaliki, south –eastern Nigeria (18), Sao Paulo Brazil (20) , Bissau, Guinea Bissau(21) and Hong Kong (22). In addition, our report of

Salmonella species (6%) is more inclined to former reports from similar studies in Bangladesh (23) and Korea .

Bacteria were isolated from 25 of 47 (53.1%) nasal swabs of children under the study. *Staphylococcus aureus* and *E. coli* were the most predominant bacterial agents, isolated in 15 (31.91%) and 10(21.3%) of the nasal swabs respectively . *Enterococcus faecalis*and *Enterobacter spp.* were isolated in only a few specimens. Few studies done previously on etiology of ARI indicate the role of *Streptococcus pyogenes* (group A β-hemolytic streptococci) in causing Acute upper respiratory infection (24). Mahmud *et al.* (25) reported bacterial isolation rate of 31.4% among the study subjects with predominant bacterial isolates such as *Staphylococcus aureus* (12.4%) and *Streptococcus pyogenes* (9.8%). Mandell (25) in another study reported *Streptococcus pyogenes* (11%) as the predominant bacteria in patients with ARI .

Bacterial infections are major causes of morbidity and mortality in children . The detection, identification, and susceptibility testing of causative species of bacteraemia is essential for the proper treatment, and better prognosis of the patient. In this study, bacteremia was confirmed in 28(56%) patient out of 50 children. the most common primary infection was identified in 50(33.3%) from 150 samples :gastroenteritis and pneumonia 47(31.3%)

The most common pathogens were *Streptococcus spp.* (35.7%) especially Beta hemolysis Streptococci mainly isolated from cases with pneumonia . Streptococcus pneumonia was also reported as the most common pathogen in children with bacteremia aged 3-36 months (27) . Pneumonia counted for 7% of these children while other respiratory tract infections counted for 24% , gastroenteritis (9%), and UTI (5%) . In another study, the source of bacteremia cases of pneumococcal infections , were pneumonia (37%) , otitis media (30%) , meningitis (11.5%), and no focal source(33%) (28,29) .

Escherichia coli was isolated in (17.9%) of the cases ; it was reported to be the most frequent gram-negative bacterial species recovered from blood cultures (9). *Klebsiella pneumonia* presented (14.3%) of the cases . In study of neonatal sepsis in Ethiopia , *Klebsiella pneumonia* was reported as the leading etiological agent (28%) (30) .

Enterococcus faecalis and *Enterobacterspp.* Were isolated from blood cultures of three children (7.14% , 3.6%) who had been hospitalized for > 10 days. Intravascular device was used during their stay , which might be a risk factor for a nosocomial infection with these species (31).

Conclusions:

From this study we can conclude the following: Diarrhoea is statistically associated with age and majority of cases occurring in children between 7months and under 5 years of age, *Escherichia coli* was isolated in (17.9%) of the cases ; it was reported to be the most frequent Gram-negative bacterial species recovered from blood cultures. The most common pathogens were *Streptococcus spp.* (35.7%) especially Beta hemolysis Streptococci mainly isolated from cases with pneumonia. There was no attempt to isolate anaerobic bacteria in this study although it might have been the cause of bacteremia in some of the cases where no aerobic bacteria were detected in blood cultures.

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