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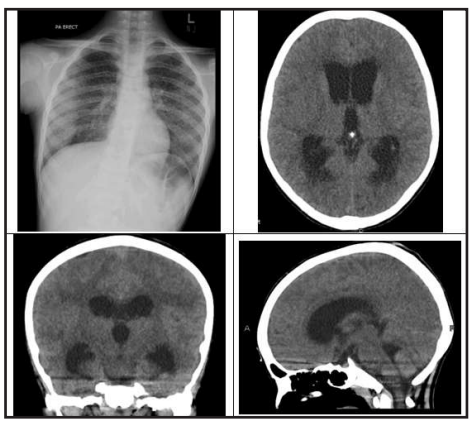

10 years old boy with fever for 1 month and drwosiness for 3 days. CXR showed miliary pattern of tuberculosis and CT head showed gross hydrocephalus. Gene Xpert (sputum and CSF) reported positive.

Courtesy: Dr Ali Faisal Saleem, Aga Khan University, Karachi.

Research Training Opportunities in Pakistan for Postgraduates

Pakistan is a Low Middle Income Country with a 2014 estimated population 193,000,000, the seventh most populous in the world. The World Bank estimates that 41 % of the population lives below the poverty line and, at $ 69 per capita spending only 2.5 % of the GDP is allocated to health. This, inevitably, has resulted in a treatment naïve population with a high communicable and non-communicable disease burden. The health infrastructural hierarchy consists of hospitals, dispensaries, rural health centres and basic health units with most resources concentrated in urban areas. In rural areas, most of the services are administered by Lady Health Workers.[1]

In 2013, public spending on health was only $9.31 per person annually, far less than the international standard $60 with almost 80% of paying for healthcare. The doctor-to-population ratio of 1:1,127 is fewer than the World Health Organisation (WHO) recommended 1:1000 and the clinical pressures intense. Fertility, child mortality and maternal death rates remain unacceptable and the high infectious and non-communicable disease burdens mean that service delivery rather than research is the overriding priority on already stretched health service providers.[2]

Punjab and Sindh are, by some distance , the most populous states the distribution reflected in numbers of medical institutions. Of the 94 medical institutions (39 public and 56 private), 48 and 23 are in Punjab and Sindh respectively. The institutions accommodate between 100 and 300 undergraduates per year and most work towards the MBBS degree, generally obtained in 5 years after which registration to the General Medical Council (PMDC) can be sought. Much of the increase in graduate numbers in recent years has been the result of the opening of private medical colleges (run along business lines) in which the quality of tuition and clinical exposure is very variable.[3]

The clinical postgraduate programme is modelled on the US one and comprises of a year as an intern (medicine and surgery) and a residency programme in the chosen subspecialty with the aim, ultimately of obtaining the FCPS exam. In those specialties in which training is recognised by the UK colleges, this can be bolstered by application to the exams for the Royal College Membership.

Were there no other pressures, many more postgraduates would augment postgraduate training with an academic fellowship as part of a research career. However, clinical load, inadequate graduate numbers and lack of central spending combine to restrict progress for many aspiring young doctors. Compounding this is the efflux of doctors from Pakistan to both the UK and US, the so called brain drain. Between 800 and 1,600 doctors are currently leaving annually and, of these, only about 15 % return.[1]

Despite this, Pakistan has a unique place in Global Health research: it has produced seminal work in areas as diverse as improving case management of pneumonia, health in pregnancy; it has developed innovations such as school based mental health and the Lady Health Worker programmes; it has produced health leaders and has been a led the way in collaborations such as the countdown to the Sustainable Development Goals and Maternal Newborn and Child Health initiatives.[4]

Though the number of research publications from medical institutions in Pakistan increased 7.5 times between 2001 and 2011, there is a huge variation in output from 2 to 521 per year, many of the manuscripts from lower output colleges appearing only in non-indexed journal (Gaffar) illustrating the huge variation in emphasis afforded to research by teaching hospitals. Against this backdrop then, how can academic work be nurtured in a country literally teeming with opportunity for high quality research with infectious disease being a particularly fertile speciality.[5]

The two main career choices for an aspiring postgraduate clinical research academic are the commercial, company sponsored route (outwith the scope of this piece) and the academic institutional one. Success in the latter route depends on a number of factors. First of these is that the infrastructure (for example supervision, statistical back up and lab facilities) is able to support robust research and award relevant degrees (MD or PhD). The second is that sufficient funding is available, a function of institutional expertise and credibility. There are a number of training grant awarding bodies such as the Fogarty fund (the developmental limb of the US NIH) and the UK Medical Research Council and Wellcome Trust but these are highly competitive and success again related to institutional experience. Career progression after acceptance onto an academic programme involves the same steps as those in the US on which the ladder is modelled: senior instructor; assistant professor; associate professor (usually requiring a PhD) and professor.

An institutionally supported sabbatical period in an HIC academic centre of excellence (like the link between AKU and Karolinska Institute in Sweden) to augment training (for example enhancing laboratory skills in infectious disease) is a path taken by many postgraduates.

Though competitive, there are now more options as a result of a number of recent changes . Trials' activity is increasing rapidly and, though many are global health related, a number of national research cooperative groups in areas such as oncology, neurology and cardiovascular science have recently been formed in which the focus has moved from observational studies to trials. The same period has witnessed a rise in contract research

organisations (CROs) with international links and improvement of the laboratory networks . What has yet to happen, at least consistently is the state registration of institutions conducting research so the best estimate of 40 centres is probably conservative. In parallel with these changes, the regulatory environment has become more rigorous. From 2002, with the recognition by the Pakistan health authorities of the ICH-GCP guidance as the accepted standard, trial protocol adherence has improved in parallel with the other innovations. The raising of these standards has, despite the well-known geopolitical instabilities, catalysed a number of multinational company sponsored studies such as the GSK supported oncology trials.[1]

Many of the higher profile academic institutes run post graduate degree programmes specifically tailored to research. Examples include the Masters in Clinical Research at the Aga Khan University, Karachi; the Clinical Research Certified Professional Programme at the Dow University of Heath Sciences; numerous Good Clinical Practice Certification courses and ethics' training offered, for example, at the Sindh Institute for urology and Transplantation and the Shaukat Memorial Cancer Centre in Lahore.

There is no doubt that, though few currently pursue this route, the research climate has changed and academic careers are now better delineated. For many, though this still requires a degree of flexibility in terms of institution and city of choice and determination to succeed, one can't help but feel that momentum has started to gather

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ORIGINAL ARTICLE

Common Infections and Antibiotic Susceptibility among Malnourished Children: A hospital based study from Karachi.

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Abstract

Background
The high prevalence of infections among children with severe malnutrition coupled with an atypical clinical presentation justifies the routine use of empirical antibiotic. The choice of antibiotics has to be guided by locally prevalent pathogens and their antibiotic susceptibility patterns. Susceptibilities of organisms keep on changingand require frequent monitoring to keep antibiotics regimen up to date.

Aim
This study is aimed at determining prevailing situation of infections and antimicrobial sensitivity of bacteria among malnourished children in peri-urban catchment area of Karachi.

Material and Method
This is hospital based retrospective analysis of children from one month to 12 years of age admitted for severe or moderate malnutrition in the pediatric ward of 'The Indus Hospital' from October 2010 till July 2011. Culture reports of blood, urine; stool, ear swab and gastric aspirate were analyzed. The Kirby-Bauer diffusion method was used to check isolates susceptibility. Commonly prescribed antibiotics were graded as sensitive or resistant according to standardized charts of Clinical and laboratory standard institute (CLSI) 2012 guidelines.

Results
Total of 260 severely malnourished children were enrolled in the study. Common infections included diarrhea 68% and respiratory tract infections 48%. Gram negative bacteria constituted 60% (61) of the total isolates, *Escherichia coli* was the leading gram negative organism (45%). Gram positive bacteria constituted 40% (38) of the total isolates .Coagulase negative staphylococci species (CONS species) 31% (31) were the most common gram positive organisms. In vitro sensitivity by disk diffusion showed 64% and 61% sensitivity to Amikacin and Gentamycin. Susceptibility of isolates to Ciprofloxacin, Amoxycillin, Co-trimoxazole and Ampicillin was 53%, 29%, 25% and 13% respectively.

Conclusion
Overwhelming resistance to WHO recommended antibiotics was seen in the study population. Diarrhea and respiratory tract infections were the commonest infections among the malnourished. E.coli was the leading organism. Most bacteria isolates were sensitive to Amikacin and Gentamicin. There is a need for re-evaluation of the WHO recommendations for management if infections in malnourished children.

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Introduction

According to national nutrition and health survey of Pakistan (NNHS) 2011, malnutrition status of children under 5 years has not shown much improvement since last 46 years in Pakistan, NNHS shows 17% wasting and 23.5% severe stunting in children less than five years of age.[1] The survey has also revealed alarming micronutrient deficiencies. Prevalence of vitamin A, vitamin D, zinc, iodine and iron deficiencies are estimated to be 56%, 41%, 36%, 33% and 24% respectively.[1]

The macro and micronutrient deficiencies lead to the deterioration of immune functions[2] which in turn lead to infections. This vicious cycle is responsible for major morbidity and mortality among the malnourished children.[3]

The high prevalence of infections among children with severe malnutrition coupled with an atypical clinical presentation of sepsis justifies the routine use of empirical antibiotic treatment in the initial phase of inpatient management as recommended by World Health Organization.[4] However, the choice of antibiotics has to be guided by locally prevalent pathogens and their antibiotic susceptibility patterns. Bacterial isolates and their susceptibilities keep on changing from time to time depending on drug usage practices, emerging drug resistance and changing health policies. Failure of monitoring current microbial trends and their drug susceptibility may lead to inappropriate treatment and possibility of drug resistance.

Previous studies have shown spectrum of common infections along with the drug susceptibility patterns in children[5,6] but either these studies are old or are not specific for malnourished children. To the best of our knowledge there are no recent local studies on bacterial isolates and their antibiotic sensitivity in undernourished children.

Therefore, this study is aimed at determining prevailing situation of infections and antimicrobial sensitivity of bacteria among malnourished children in peri-urban catchment area of Karachi.

Objective

This study is aimed at determining prevailing situation of infections and antimicrobial sensitivity of bacteria among malnourished children in peri-urban catchment area of Karachi.

Patients and Methods

This is hospital based retrospective analysis of children from one month to 12 years of age admitted for severe or moderate malnutrition in the pediatric ward of Indus Hospital from October 2010 till July 2011.Indus is a 150 bed hospital located in Korangi, Karachi. It serves population of 2.5 million people. The main catchment areas consist of peri urban settlements and fisherman villages. It shoulders a large share of treating malnourished children along with public sector hospitals of Karachi.

Table 1: Characteristics of 250 malnourished children admitted in pediatric ward of Indus Hospital.

	Median (IQR)	
Age in years	2.36 (1.77-3.17)	
Hospital stay (days)	5 (3-7)	
	N	**%**
Gender		
Male	132	53
Female	118	47
Age groups		
<1year	7	2.8
>1 year	243	97
Acute respiratory infection (ARI)	120	48
Otitis media	7	3
Oral thrush	55	22
UTI	14	5.6
Malaria	8	3.2
Diarrhea		
Overall	169	68
Bacterial	39	23
Protozoal	6	3.5
Tuberculosis		
Overall	17	
Pulmonary	10	59
Abdominal	5	29
Nodal	2	11

Data was extracted from records of electronic information system (HMIS) of Indus Hospital. Duration of hospitalization was defined as the duration from the date of admission in hospital to the date of discharge, death or transfer to another hospital, or the date when patients left hospital against medical advice.

Inclusion and Exclusion Criteria

Children with severe acute malnutrition (SAM) and moderate acute malnutrition (MAM) were included in the study. Children who had chronic illnesses like chronic renal failure, congenital heart disease and immunodeficiency syndrome or those children who had received antibiotics prior to enrollment were not included.Study participants who did not complete the study were excluded.

Methods

A predesigned proforma was used to collect relevant information. The information included demographic and nutritional details of malnourished children. The laboratory information included microscopic/ culture reports of blood, urine, stool, ear swab and gastric aspirate. Results of imaging studies and drug sensitivity analysis of isolated organisms were also included.

Approval from the ethical review board of the hospital was taken prior to starting the study. Confidentiality of subjects and privacy of data was maintained throughout the study. Data was stored on Microsoft Excel sheets. Each study participant was allotted a study number which was not linked to any of the participants' personal identifiers. At the end of data collection, remaining identifiers, like names, addresses, medical registration numbers, lab or radiology report numbers, were permanently deleted from the data sheets. The data files were password protected and were only available to study investigators. There is no known conflict of interest in this study.

Moderate acute malnutrition (MAM) and severe acute malnutrition (SAM) were categorized according to WHO guidelines.[4] SAM was classified if mid upper arm circumference (MUAC) is less than 11.5cms or there is bilateral pedal edema or weight for height ratio is <3 z-scores of the WHO child growth standard, MAM included children having MUAC between 11.5–12.5 cms or weight-for-height between –3 and –2 z-scores of the World Health Organization.

Laboratory Evaluation

All the lab specimens used in the study were collected under aseptic conditions using standard procedures. For blood culture 1-3 milliliters of blood was drawn from a peripheral vein under aseptic conditions.The skin was cleaned with chlorhexidine and povidone iodine solution before drawing blood. Each blood sample was then inoculated into BACTEC Paeds plus F culture vials containing Soybean-Casein digest broth with resins, the sample was incubated at 37°C for 24 hours in the automated system, after which bottles were observed for turbidity. From

Tables 2: Bacterial isolates and their site of infection.

	Site of infections N (%)				
Bacteria	**Blood**	**Urine**	**Stool**	**Ear**	**Total**
Gram +ve organisms: 38 (38)					
CONS	31	-			31 (81)
MRSA	4	-		2	6 (11)
Streptococcus	3	-			3 (8)
Gram –ve organisms: 61 (62)					
E.coli	3	4	39		46 (75)
Pseudomonas	1	1		2	4 (7)
Salmonella	3	-			3 (5)
Klebsiella	-	5		1	6 (10)
Proteus	-	-		2	2 (3)
Total	45	10	39	7	101

Table 3: Susceptibility pattern of Gram negative bacterial isolates to commonly selected antibiotics

Antimicrobials	**Drug susceptibility of bacterial isolates** Susceptibility/Total (%)				
	E. coli (46)	**Salmonella** (3)	**Klebsiella** (6)	**Proteus** (2)	**Pseudomonas** (4)
Amikin	25/29(86)	3/3(100)	5/6(83)	½ (50)	¾ (75)
Amoxcillin	8/43(18.6)	3/3(100)	2/3(66)	N/D	All strains resistant
Ampicillin	3/45(6.6)	1/3(33)	N/D	N/D	All strains resistant
Ceftazidime	2/38(5.2)	3/3(100)	2/3(66)	½ (50)	All strains resistant
Ciprofloxacin	22/45(48.8)	3/3(100)	4/6(66)	½ (50)	N/D
Co-trimoxazole	12/45(26.6)	2/3(66)	¾ (75)	N/D	All strains resistant
Vancomycin	N/D	N/D	N/D	N/D	N/D
Gentamycin	29/44(66)	3/3(100)	3/3(100)	1/1(100)	4/4(100)
Nitrofurantoin	5/11(45)	N/D	1/1(100)	N/D	N/D
Tobramycin	N/D	N/D	N/D	N/D	¾ (75)

E.coli= Escherichia coli, MRSA= Methicillin resistant Staphylococcus Aureus, CONS= Coagulase negative staphylococci, Salmon=Salmonella Typhimurium, Strep=Streptococcus pneumonia, Klebs= Klebsiella pneumonia, Proteus=Proteus mirabilis, Pseudomonas= Pseudomonas aeruginosa.

bottles showing turbidity, Gram's stain was done and further inoculations were made on blood, chocolate, MacConkey and Sabourand-dextrose agar respectively. The plates were then incubated at 37°C for 18 – 24 hours. Culture bottles that did not show turbidity were further incubated for up to 10 days. Identification of Staphylococcus aureus was done by Coagulase test.

The Kirby-Bauer diffusion method was used to test the susceptibility to the isolates on Muller-Hinton Agar according to Clinical and laboratory standard institute (CLSI) 2012 guidelines.[7] Commonly prescribed antibiotics were tested and graded as sensitive or resistant according to zone sizes which

Table 4: Susceptibility pattern of Gram positive bacterial isolates to commonly selected antibiotics

Antimicrobials	Drug susceptibility of bacterial isolates Susceptibility/Total (%)		
	CONS(31)	**MRSA**(6)	**Streptococcus**(3)
Amikin	26/27(96)	N/D	2/2(100)
Amoxcillin	10/28(35.7)	4/6(66)	2/2(100)
Ampicillin	6/22(27)	2/6(33)	1/3(33)
Ceftazidime	N/D	N/D	N/D
Ciprofloxacin	18/25(72)	4/6(66)	2/3(66)
Co-trimoxazole	5/23(21.7)	2/6(33)	1/3(33)
Vancomycin	27/27(100)	3/3(100)	N/D
Gentamycin	16/26(61.5)	4/5(80)	2/3(66)
Nitrofurantoin	N/D	1/1(100)	N/D
Tobramycin	3/5(60)	N/D	N/D

E.coli= Escherichia coli, MRSA= Methicillin resistant Staphylococcus Aureus, CONS= Coagulase negative staphylococci, Salmon=Salmonella Typhimurium, Strep=Streptococcus pneumonia, Klebs= Klebsiella pneumonia, Proteus=Proteus mirabilis, Pseudomonas= Pseudomonas aeruginosa.

are interpreted according to standardized charts of CLSI-2012. Clean-voided midstream or catheterized urine specimens were taken for urine analysis. Urine microscopy and culture was done according to standard procedures at 24 and 48 h. Growth was categorized as negative when colony count of bacteria was $<10^4$ CFU/ml and positive when colony count was $>10^5$ CFU/ml. Organisms species identification was done by standard biochemical tests.

Diagnosis of Malaria was made by microscopic examination of thick and thin film of Giemsa stained blood films. Thick film was used for estimation of parasite load and thin film was done for species identification.

Stool sample analysis consisted of direct microscopy and Iodine staining for parasites, stool cultures was done on MacConkey and Salmonella-Shigella (SS) agar. Where clinically suspected, Thiosulphate-Citrate-Bile-Sucrose (TCBS) agar was done for vibriocholera.

Gastric aspirates were used for isolation of Mycobacterium Tuberculosis. Cultures for tuberculosis consisted of both solid and liquid mediums. 7H9 broth constituted the liquid medium while Lowenstin Jenson medium was used as solid agar. Drug sensitivity testing for first line anti-tuberculous drugs was also done on Mycobacterium growth indicator (MGIT).

Statistical analysis

Data was entered and analyzed using SPSS 21. Descriptive analysis was done. Median (IQR) was calculated for age of patient (years) and hospital stay (days). Frequency and percentage was computed for baseline characteristics of malnourished children gender, pneumonia, malaria, otitis media, UTI, diarrhea, TB and oral thrush. Rate of susceptibility was computed for commonly selected antibiotics of bacterial isolates.

Results

Total of 260 severe and moderate malnourished children were enrolled in the study, 10 participants were excluded from the final analysis due to incomplete data. Fifty three percent children were males while 47% were females. The median age was 2.36 years (IQR 1.77-3.17). Majority of the children (97%) were above one year of age. Un-vaccinated, partially and completely vaccinated children were 16% (19/118), 21% (25/118) and 63% (74/118) respectively. Commonly diagnosed infections included diarrhea 68% (based on clinical exam and positive cultures) and respiratory tract infections 48% (positive x-ray findings). Seventeen children were suffering from tuberculosis out of which 10 (58%) had pulmonary tuberculosis and 7 (41%) had extra pulmonary tuberculosis (EPTB). Abdominal and nodal T.B was present in 5/7 (29%) and 2/7 (11%) children. The common laboratory findings among the tuberculosis children were hilar lymphadenopathy (13/17) and chest cavities (8/17). Manteaux test was positive in 4 out of 17 tuberculous children, 2 out of 17 samples of gastric aspirate grew Mycobacterium Tuberculosis. Three out of five children with abdominal T.B had ascites, matted abdominal loops and abdominal lymphadenopathy. Eight children had malaria out of which 6 had plasmodium Vivax and 2 had plasmodium Falciparum infections.

One hundred and one (40%) of the 250 specimens cultured, grew bacterial isolates. Gram negative bacteria constituted 60% (61) of the total isolates, *Escherichia coli* was the leading gram negative organism (45%) followed by Klebsiella (6%) and Pseudomonas Aerouginosa (4%) species.Gram positive bacteria constituted 40% (38) of the total isolates. Coagulase negative staphylococci species (CONS species) 31% (31) were the most common gram positive organisms followed by Methicillin resistant staphylococcus aureus (MRSA) 6% (6) and streptococcus 3% (3).

In vitro sensitivity by disk diffusion showed that 64% organisms were sensitive to Amikin, 61% organisms were sensitive to Gentamycin. Susceptibility of isolates to Ciprofloxacin, Amoxycillin, Co-trimoxazole and Ampicillin was 53%, 29%, 25% and 13% respectively. All the strains of MRSA and CNS species were resistant to Cloxacillin.

Discussion

Relation between malnutrition and infection is bidirectional ending up in a vicious cycle. Infections cause hyper metabolism in malnourished children which leads to further weight loss due to reduced food intake and increased excretion of nitrogen.[8,9]

The common infections observed in nutritionally deprived

children are gastrointestinal and respiratory infections. The first line of defense against these types of infection is the innate immune system, particularly epithelial barriers and the mucosal immune response. Mucous production is significantly reduced in intestinal and respiratory tracts of malnourished children resulting in loss of protective mucus blanket which normally sweeps away bacteria[10] Secretory IgA another important component of the mucosal response is also reduced making malnourished children more prone to develop diarrhea and respiratory tract infections.[11]

In our study predominant infection was diarrhea (68%) followed by respiratory infections (48%).This is consistent with other studies.[12,13,14] Excessive diarrhea can be explained by presence of atrophied and permeable gut mucosa, poor hygienic conditions and Zinc and vitamin A deficiencies among malnourished children. Excessive gut colonization with bacteria and yeast can also be a contributing factor.[15] All cases of diarrhea were caused by *Escherichia coli* (*E.coli*) in our study which is similar to another study done in a tertiary hospital of Karachi in 2010.[16] This could be due to contamination of food and water and unsatisfactory hygienic conditions. A study from Bangladesh found contamination of food and water with *E.coli*.[16] Education of mothers in food safety measures and improving water supply and sanitation can reduce bacterial diarrhea to a great extent in malnourished children.

Bacterial pathogens were isolated from 12 out of 120 cases of pneumonia; *Methicillin resistant staphylococcus aureus* (MRSA), *Streptococcus pneumonia* and *Klebsiella* were responsible for 6, 3 and 3 cases respectively. Other studies have reported *Streptococcus pneumonia and Haemophilus influenzae* as main causative agents of pneumonia in malnourished children.[17] Lack of similar finding in our study could be due to increased use of pneumococcal and *H. influenzae*type b vaccines in Pakistan.

Most of the infections were caused by gram negative bacteria (61%). The most common organism was *Escherichia coli* (45%) followed by Klebsiella 6%) and Pseudomonas Aerouginosa (4%). Reduced function of complement system, diminished chemotactic and phagocytic abilities of leucocytes could be the reasons of predominant gram negative infections.[18,19]

Gram positive bacteria constituted 40% (38) of the total isolates. Coagulase negative staphylococci species (CoNS species) 31% (31) were the most common gram positive organisms followed by Methicillin resistant staphylococcus aureus (MRSA) 6% (6). Presence of CoNs has been reported in other studies as well.[5,20]

CONS form part of the normal skin flora, and are frequent contaminants of blood cultures but rarely cause significant infections in immune-competent hosts. To differentiate between CoNS-positive blood cultures due to skin contamination from a true blood stream infection is difficult. Clinicians often rely on presence of systemic signs of bacteremia along with a positive culture for treatment. Such assessments have not been reported for malnourished children. Currently CONS is regarded as contaminant in malnourished children. In our study both Chlorhexidine and povidone iodine solutions were used to disinfect the skin prior to blood sampling in order to minimize risk of skin contamination. We gave antibiotics to children who had clinical signs of bacteremia along with CONS positive blood culture due to the reason that many children had skin ulcers making them prone to invasive CONS infections. Also it was clinically difficult to differentiate true infections from refeeding syndromes and silent aspirations. In our opinion judging clinical significance of CONS among malnourished children is vital as it will have profound impact on future treatment guidelines. We strongly believe that more studies in this regarded are needed.

In our study sensitivity to Amikacin was 64%, sixty one percent of organisms were sensitive to Gentamycin while 53% Susceptibility of organisms to Ciprofloxacin was noted. Deceased susceptibility was documented to commonly used antibiotics, such as Amoxicillin, Co-trimoxazole and Ampicillin. Similar resistance to WHO recommended antibiotics have been noted in other studies also.[21,22]

WHO recommends ampicillin parenterally for 2 days followed by enteral amoxicillin/ampicillin for a further 5 days and gentamicin parenterally for 7 days.[23] WHO guidelines also do not differentiate antibiotic treatment for hospitalized severe acute malnutrition children (SAM) with those who have danger signs such as sepsis, cyanosis, grunting, convulsion and inability to drink. Furthermore there is a lack of studies addressing toxicity of Gentamicin in terms of renal function and otoxicity in SAM children.

Keeping in mind that microbial resistance profiles vary widely with time and also introduction of pneumococcal and *Haemophilus influenzae* type b vaccines in Pakistan is expected to change spectrum of pathogens we recommend that clinicians should choose initial antibiotics according to current local microbial profile and susceptibility patterns until a change is warranted based on culture sensitivity report or clinical deterioration of the patient. No recent efforts have been made to audit current situation of antibiotic sensitivity and the magnitude of bacteremia in malnourished children and a recent survey in this regard is urgently needed.

Limitation

We were unable to evaluate children for HIV status and repeat blood cultures for CoNspositive cases to rule out contaminants from true bacteremia. This could affect the outcomes.

The WHO recommendations for management of infections in malnourished children is followed in many regions, however,

this study has only evaluated results from a single center in one country.

Conclusion

Overwhelming resistance to WHO recommended antibiotics was seen in the study population. Diarrhea and respiratory tract infections were the commonest infections among the malnourished.Gram negative infections were predominant and E.coli was the leading organism. Most bacteria isolates were sensitive to Amikacin and Gentamicin. There is a need for re-evaluation of the WHO recommendations for management if infections in malnourished children.

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ORIGINAL ARTICLE

Non-Acetaminophen Induced Acute Liver Failure of Viral etiology: Treatment with and without N-Acetylcysteine; comparing the length of hospital stay and survival status in children at the tertiary care hospital

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Abstract

Background
Acetaminophen poisoning is a common cause of acute liver failure (ALF) and is treated with N-Acetylcysteine (NAC), which acts as an antidote, antioxidant and anti-inflammatory agent. NAC role in non-acetaminophen induced acute liver failure among children remains controversial and a few centers have adopted this option. Viral hepatitis (A and E) remains the leading cause of ALF in Pakistan. We aim to determine the role of NAC in ALF secondary to viral etiology.

Methodology
We performed this quasi experimental study at National Institute of Child Health, Karachi from December 2013 to December 2014**.** All Children of either gender between ages 5 to 13 years presented with viral induced ALF were enrolled. Children treated with NAC were included in group A and children not given NAC were enrolled in group B. NAC was administered as a continuous intravenous infusion (100 mg/kg/24 hours) until normalization of the INR or death. Standard care treatment was similar throughout the study period. The two groups were compared for the length of hospital stay, discharged or death.

Results
There were total 32 patients with 22 males and 10 females. Causes of ALF were HAV 23 (72%), Non A to E 5 (16%), HBV 3(9%) and both A and E in a patients. In group A, all 16 patients received NAC for median duration of 15.5 days. Length of hospital stay in group A was 14.4±6.7 days (median 15.5, IQR(11): 7.5 days to 18.5 days) while in group B it was 23.8±4.1 days (median 24, IQR(8): 19.5days to 27.5 days) p-value 0.001. Survival was higher in those who received NAC 11 (69%) than those who did not receive 7 (44%) but there was no statistically significant relationship was observed (p-value 0.154).

Overall mortality was found 44% (14 expired out of 32 patients).

Conclusion
There was no significant difference in overall survival of patients treated with NAC and without NAC. There was a significant improvement in survival observed in patients with early stage of encephalopathy grade 1& 2 treated with N-Acetylcysteine.

Keywords
Length of hospital stay, Survival status, N-Acetylcysteine, Acute liver failure

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Introduction

Acute liver failure (ALF) is a relatively uncommon but life threatening syndrome.[1] It is considered a medical emergency because of its multisystem involvement and association with high mortality rate.[2] The currently accepted definition in children includes biochemical evidence of acute liver injury (usually <8 wk duration), no evidence of chronic liver disease, and hepatic based coagulopathy defined as prothrombin time (PT) >15sec or international normalized ratio (INR) >1.5 not corrected by vitamin K in the presence of clinical hepatic encephalopathy, or PT >20 sec or INR >2 regardless of clinical presence of hepatic encephalopathy.[3]

Intentional or unintentional acetaminophen poisoning has recently been evolved as most common etiology of acute liver failure in developed countries while in under developed countries; the most common etiology of ALF has been reported as viral and hepatitis A has been found as most frequent cause.[4,5,6] Published data regarding ALF in pediatric population from Pakistan reported viral etiology constitute approximately 75% of cases and reported mortality was in more than 50% of cases.[7,8] There is no specific treatment for ALF and supportive treatment is targeted to make patient hemodynamically stable, correcting coagulopathy, prevention or treatment of cerebral edema, coma care in unconscious patient and management of fluid, electrolytes and acid-base imbalances. Spontaneous recovery has been reported in 40% to 56% of pediatric patients with supportive treatment in different studies.[7,9]

Acetaminophen poisoning causing ALF is frequently treated with N-Actylcysteine (NAC), which acts as an antidote, an antioxidant and an anti-inflammatory agent. There was a study that suggested that NAC could also help children with non-

acetaminophen induced acute liver failure (NAI-ALF) by improving survival status and shortening hospital stay[10] which has led some medical centers to adopt this treatment modality.

The outcome of patients with ALF has been dramatically improved with liver transplantation.[11,12] In developing and underdeveloped countries this option is available only at few centers so we have to search for other modalities of treatment because in our part of world even at tertiary care set up liver transplantation facility is not available.[13]

Sotelo N *et al* described beneficial effect of NAC in NAI-ALF of viral etiology.[14] A recent retrospective single-center analytical study in children from Pakistan concluded that NAC showed improvement in LFTs and encephalopathy.[15] There is lack of prospective studies in treatment of ALF with and without NAC from developing countries. The objective of this study is to compare the length of hospital stay and survival status in children with and without NAC.

Methodology

A Quasi experimental study conducted at National Institute of Child Health, Karachi over a period of 1 year from December 2013 to December 2014. Sampling technique was non-probability consecutive sampling. Children of either sex, between the ages of 5 to 13 years, presenting with non-acetaminophen induced ALF of viral etiology, admitted to pediatric unit of NICH were included in the study. Children presenting with acetaminophen induced ALF or other etiologies like autoimmune hepatitis and Wilson disease were excluded from the study.

The acute liver failure was defined as presence of biochemical evidence of acute liver injury (usually <8 week duration), no evidence of chronic liver disease, prothrombin time (PT) >15sec or international normalized ratio (INR) >1.5 not corrected by vitamin K in the presence of clinical hepatic encephalopathy, or PT >20 sec or INR >2 regardless of clinical presence of hepatic encephalopathy. The outcome was measured at the hospital discharge in terms of survival and duration of hospitalization. Survival was defined as discharge of patients from hospital from same admission and duration of hospitalization was defined as total duration of hospital stay from the day of taking N-Acetylcysteine.

An approval from local ethical committee was granted and a written informed consent was taken from the parents / guardians of the child. Clinical history was taken by the principal investigator. Age, sex, duration of stay in hospital, durations

of symptoms and duration between onset of initial symptoms and onset of encephalopathy, clinical signs and stage of encephalopathy were recorded.

Investigations including liver function test and prothrombin time (PT) were carried out. For etiology, viral markers for hepatitis A, B, C & E (anti HAV IgM, HBsAg, anti HCV antibody, anti HEV IgM) were done. The diagnosis of non-A to E hepatitis was made in cases with clinical presentation suggestive of viral etiology in the absence of any viral marker positivity and autoimmune hepatitis and Wilson diseases work up was negative. Subjects were randomly divided into study groups A and B by using lottery method. Group A included children routinely treated with NAC and Group B was of children not treated with NAC. NAC was administered as a continuous intravenous infusion (100 mg/kg/24 hours) until normalization of the INR or death. Standard care treatment was similar throughout the study period. This included continuous intravenous dextrose infusion to prevent hypoglycemia; broad spectrum prophylactic antimicrobials to prevent bacterial and fungal infections and antacids to prevent acute gastrointestinal bleeding. Children were admitted to the pediatric intensive care unit if they developed encephalopathy grade 2, became hypoxic, or needed sedation. Fresh frozen plasma was given only if an invasive procedure needed to be done. Infection was diagnosed only when a pathogenic microorganism was detected in blood, urine or tracheal cultures. The two groups were compared for the length of hospital stay, discharged or death. All the demographic and clinical data with laboratory finding and short term outcome after administration of NAC was collected on a predesigned proforma by the principle investigator.

Data was compiled and analyzed into SPSS 17. Mean ± SD was calculated for quantitative variable that is age and length of hospital stay, frequency and percentage was calculated for gender and survival. Median, IQR, Two sample‘t’ test was applied to compare length of the hospital stay in both groups, $p<0.05$ was taken as significant. Chi square test was applied to compare survival in both groups, taken $p<0.05$ as significant.

Results

Of total 32 patients,[16] patients were in each group i.e group A comprised of patients that were treated with NAC and group B patients did not receive NAC. There were 22 males and 10 females with 10 males and 6 females in group A and 12 males and 4 females in group B. Table 1 describes the clinical characteristics of all patients.

Mean age of the patients was 7.5±1.36 years in group A and 7.6±1.23 in group B. The minimum age of the patients was 5.5 while maximum age was 9.5 years.

Etiologies of ALF were HAV 23(71.9%), Non A to E 05 (15.6%), HBV 03(9.4%) and Combined A & E 01(3.1%) patients. As shown in table 1.

In group A, all 16 patients received NAC for median duration of 15.5 days.

The clinical features recorded in patients presenting with ALF were fever 21 (65.6%), loss of appetite 25 (78.1%), Nausea & vomiting 25 (78.1%), Abdominal pain 14 (43.8%), Jaundice 29 (90.6%), Hepatomegaly 23 (71.9%), Bleeding 10 (31.3%).

Regarding encephalopathy in group A patients presenting with grade I were 5(15.6%), grade II 7(22%), grade III 3(9.4%) and Grade IV 1(3.1%) while in group B grade I 6(18.7%), grade II 4(12.5%) , grade III 4(12.5%) and grade IV 2(6.2%). Seven patients in group A and 5 patients in group B developed encephalopathy within 7 days of illness (hyperacute) while 9 in group A and 11 in group B developed encephalopathy after 7 days of onset (acute). In terms of grade of encephalopathy and time of development of encephalopathy there was no statistically significant difference in group A and group B as shown in table 1.

Regarding investigation Total Bilirubin was 7.9±5.2 mg/dl in group A and 7.9±4.5 mg/dl in group B, AST was 1360.4±540.2 in group A and 1405.6±467.6, ALT was 1401.9±612.0 in group A and 1446.2±526.8 in group B, Prothrombin Time (PT) was 50.8±9.4 in group A and 54.7±7.1 in group B, Albumin was 2.744±0.333 in group A and 2.650±0.398 in group B, Serum potassium (mEq/L) was 3.818±0.616 in group A and 3.85±0.636 in group B, Serum creatinine (mg/dl) was 1.031±0.707 in group A and 1.2±0.791in group B and Platelets (per microliter) was 224812±123933 in group A and 203937±151614 in group B,. All the values are indicating mean ± standard deviation and P value shows no statistically significant difference in both the groups except PT as clear from table 1.

Length of hospital stay in group A was 14.4±6.7 days (median 15.5, IQR(11): 7.5days to 18.5days) while in group B it was 23.8±4.1days (median 24, IQR(8): 19.5days to 27.5days) p-value 0.001 as shown in table 2.

Overall mortality was found 43.8% (14 expired out of 32 patients).

Survival status of both groups was compared to see the effect of treatment. In group A 11(69%) patients survived and in group B 7(44%) patients survived. Survival was higher in those who received NAC 11(69%) than those who did not receive NAC 7(44%) but there was no statistically significant difference from evidence shown in table 3.

Discussion

Acute liver failure of various etiologies is not a rare clinical syndrome resulting in sudden necrosis of hepatocytes causing encephalopathy and coagulopathy. Viral hepatitis is reported as the most frequent etiology of acute liver failure from developing countries. Advancement in critical care have

Table 1: Demographic, Etiology, Clinical features and investigations in both groups A & B

	Child with NAC in treatment	Child without NAC in treatment	P–Value	C. I.
Age (Mean ±SD)	7.5±1.366	7.6±1.232	0.615	(-0.644, 0.394)
Male	10	12	0.4456	
Etiologies of ALF			total	percentage
HAV	10	13	23	71.9
Non A-E	3	2	5	15.6
HBV	2	1	3	9.4
Co infection HAV & HEV	1	0	1	3.1
CLINICAL FEATURES				
Fever	10 (31.3)	11 (34.4)		
Loss of appetite	12 (37.5)	13 (40.6)		
Nausea & Vomiting	12 (37.5)	13 (40.6)		
Abdominal Pain	8 (25)	6 (18.8)		
Jaundice	13 (40.6)	16 (50.0)		
Hepatomegaly	11 (34.4)	12 (37.5)		
Bleeding Manifestation	5 (15.6)	5 (15.6)		
HEPATIC ENCEPHALOPATHY				
I	5 (15.6)	6 (18.7)	0.709	
II	7 (22)	4 (12.5)		
III	3 (9.4)	4 (12.5)		
IV	1 (3.1)	2 (6.2)		
ONSET OF ENCHEPHALOPATHY AFTER ONSET OF INITIAL SYMPTOMS				
< 7 Days (hyperacute)	7	5	0.4652	
INVESTIGATIONS				
Total Bilirubin (mg/dl)	7.9±5.2	7.9±4.5	0.892	(-1.352, 1.540)
Direct Bilirubin (mg/dl)	6.1±4.3	6.6±4.2	0.334	(-1.470, 0.532)
AST	1360.4±540.2	1405.6±467.6	0.422	(-162.2, 71.7)
ALT	1401.9±612.0	1446.2±526.8	0.442	(-163.6, 75.1)
Prothrombin Time	55.00±14.41	61.56±14.21	0.2779	(-18.98, 5.86)
Albumin	2.744±0.333	2.650±0.398	0.5636	(-0.245,0.432)
Serum Potassium (mEq/L)	3.819±0.617	3.85±0.637	0.908	(-0.599,0.537)
Serum Creatinine (mg/dl)	0.969±0.679	1.2±0.792	0.419	(-0.824,0.361)
Platelets (/ul)	224813±123934	203938±151615	0.701	(-92815,134565)

Table 2: Comparison Of Groups For Length Of Hospital Stay

Parameters	Mean ±SD	P-value	95% C.I
Group A	14.4±6.7	0.001	(-14.00, -4.75)
Group B	23.8±4.1		

improved overall outcome of ALF, however in advanced cases the definitive therapy is liver transplantation.[16] In our country the liver transplantation facility is not easily available. Rapidly progressive course and associated high mortality of ALF of viral etiology in previously normal children demand alternative treatment. If the cause of ALF is acetaminophen NAC should be administered as soon as possible. But in a study retrospective review of medical records of 170 children presenting with non-acetaminophen induced ALF between 1989 and 2004 was

Table 3: Treatment Groups & Discharge disposition

Group	Survival		Total	d.f	Chi-Square	P-Value
	Yes	No				
A	11	5	16			
B	7	9	16	1	2.03	0.154
Total	18	14	32			

undertaken who were given NAC, which showed decrease hospital stay and better survival in these patients. Although the short comings of the study was it was uncontrolled, retrospective and on a small number of patients.[10]

In a prospective, double-blind trial total of 173 adult patients received NAC (n=81) or placebo (n=92), 20% of patients were of viral etiology. Overall survival was 70% for patients given NAC and 66% for patients given placebo. Transplant-free survival was significantly better for NAC patients (40%) than for those given placebo (27%). Intravenous NAC generally was well tolerated; only nausea and vomiting occurred significantly more frequently in the NAC group (14% vs 4%).1 We found similar results in our cohort for length of hospital stay. Furthermore, comparison was done in our study to see the effect of treatment group on the survival status. Survival was higher in those who received NAC as compared to those who did not receive NAC treatment for ALF.

Further analysis for survival status according to grade of encephalopathy, we found that all patients with encephalopathy grade 3 & 4 expired in both groups and there was statically significant evidence was found in patients with hepatic encephalopathy grade 1 & 2, that who received NAC had higher survival than Non-NAC group. Similar results were reported by WM Lee *et al* that coma grade 1 & 2 receiving NAC found to have significantly higher survival than coma grade 3 & 4 receiving NAC.[1] Sotelo N found early treatment with NAC is safe and effective alternative treatment for acute liver failure of viral etiology.[14] A study from Pakistan also reported death of all patients with grade 4 coma.[15] Results from above studies indicates that NAC augments recovery in some earlier stages of encephalopathy beyond that perhaps significant irreversible damages to body organ occur that have less response to this drug that may be evaluated in further studies.

The limitation of our study is that it is a single center study on small number of patients. Efficacy of NAC may be further evaluated in studies with focus on early administration in grade 1 & 2 of encephalopathy with randomized controlled trails on larger number of patients.

Conclusion

Most common etiology of ALF was found Hepatitis A. There was no significant difference in overall survival of patients treated with NAC and without NAC. There was a significant improvement in survival observed in patients with early stage of encephalopathy grade 1& 2 treated with N-Acetylcysteine.

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Probiotics in Health and Disease in Children

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Abstract

Probiotics are living microorganisms which are being used in the management of multiple medical conditions associated with disturbance of normal microflora of human mucosa. The expected benefits and potential risks of probiotics use have been evaluated and guidelines have been suggested for their safe and effective use in hospitalized and ambulatory pediatric patients. If minimal benefit is expected then financial cost should also be taken into account. Research is going on to develop probiotics by isolating the beneficial component of the microbe, and thus decrease the potential risks of using live microorganisms.

Introduction

Any physician who prescribes antibiotics is familiar with antibiotic associated diarrhea. The incidence of Clostridium difficile associated diarrhea is increasing.[1] Change in gut microflora due to antibiotic therapy can contribute to post infectious diarrhea, not associated with *C.difficile*. Systemic antibiotics, whether given orally or parenterally will alter the normal microflora of the human gut by decreasing antibiotic susceptible bacteria in the microbiome. It is only in the last decade that we have understood the mechanism of the antibiotic associated diarrhea and how the commensal microbes maintain the integrity of the gut mucosa, decrease the paracellular permeability induced by pathogens and inhibit proinflammatory cytokines.[2]

Systemic antibiotics as well as local application, such as antibiotic eye drops, antibiotic cream applied to nares or nebulized antibiotics will alter the microflora of the oropharynx and respiratory tract. The vaginal microflora is similarly changed by systemic antibiotics.

In many instances, as antibiotics decrease the commensal bacteria, Candida which is part of normal flora, overgrows leading to oral thrush, vaginitis, esophagitis, intertriginous dermatitis and diaper rash.

These everyday clinical observations have encouraged many clinicians to use probiotics. Generally probiotics are living microorganisms but components of microbial cells that have a beneficial effect on human host are also called probiotics. Probiotics are often used with prebiotics; these are substances which enhance the proliferation of probiotic microorganisms. These include fructo and galacto oligosaccharides, inulin, germinated barley and psyllium. These substances pass unchanged through upper GI tract. Combined probiotics and prebiotics are called synbiotics.[2]

The United Nations Food and Agriculture Organization (FAO) and World Health organization (WHO) have defined probiotics as "live microorganisms, which when administered in an adequate amount confer a health benefit on the host."[3]

Objective

This review aims to evaluate present evidence which is often contradictory and many times incomparable due to use of different microbes, different species, and different subspecies and number of CFU used. The preparations available in Karachi, their components, experimental and clinical data supporting their use, possible drawbacks and usual price will also be considered, without supporting any particular brand.

Background

A standard Pediatric textbook, Nelson Textbook of Pediatrics, 18th edition, published in 2007, has a comprehensive chapter: Probiotics in Gastrointestinal Diseases by David Branski and Michael Wilschanski of Hebrew University Medical School, Israel. This chapter refers to the basic science of immunomodulation by probiotics as well as clinical studies in childhood diarrheal and atopic disorders. It addressed lactic acid producing bacilli, Lactobacillus and Bifidobacterium and the yeast Saccharomyces boulardii and reiterated that as probiotic therapy introduces living microbes in a person's body it should meet certain criteria for safety and efficacy (Table1,2,4). After discussing the clinical studies of probiotic use in acute infectious diarrhea, antibiotic associated diarrhea, Neonatal Necrotizing Enterocolitis (NEC), Lactase deficiency, Irritable Bowel Syndrome, Inflammatory Bowel disease, Celiac disease, food protein sensitivity and Helicobacter pylori infection; the chapter ended with exhortation to define the beneficial strains and optimal combinations of strains. It raised concerns regarding transfer of virulence and antibiotic resistance. as well as safety in immune deficient infants and possible induction of dental caries.[2]

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Table 1: Criteria for microorganisms to be used as Probiotics

1. It should be of human origin.
2. It should be nonpathogenic and safe.
3. It should resist digestion by gastric and pancreatic juices and bile.
4. It should adhere to enterocytes.
5. It should produce antimicrobial substances.
6. It should produce favorable immunomodulation.
7. It should be able to influence metabolic activities.

Table 2: Commonly used Probiotic Microorganisms for Prevention and Treatment of Disease

BACTERIA

Lactobacillus
L. acidophilus
L. bulgaricus
L. caseii
L. fermentum
L. paracasei
L. plantarum
L. rhamnosus (GG)

Bifidobacterium
B. bifidum
B. breve
B. infantis
B. lactis
B. longum

Streptococcus
S. boulardii
S. salivarius
S. thermophilus
Lactococcus lactic
Enterococcus faecium SF68 (nonpathogenic)
Escherichia coli Nissle 1917 (nonpathogenic strain)
Clostridium butyricum

Yeast
Saccharomyces boulardi

From Feign and Cherry's Textbook of Pediatric Infectious Diseases. 7th edition,2014.
Harrison GJ. Probiotics chapter 242, Page 3370.

The 20th edition of Nelson Textbood of Pediatrics published in 2016 discusses probiotics in the chapter: The Microbiome and Pediatric Health, and supports probiotic use in antibiotic associated diarrhea, reduction and recurrence of C. difficile infection and prevention of NEC. Probiotics were deemed to be of possible use in recurrent Urinary tract infections and respiratory infections. There do not seem to be clear guidelines or strong endorsement despite intervening nine years of clinical use.[4]

Clinical Use of Probiotics
In clinical use probiotics have been considered from three aspects[1]

1. Role in suppressing the pathogenic bacteria.
2. Role in improving intestinal barrier function.
3. Role in development and modulation of immune system.

Gastrointestinal Disorders
Gastroenteritis (GE) remains a leading cause of disease worldwide. The role of probiotics in suppressing intestinal pathogens and improving the barrier function of lining enterocytes (by decreasing paracellular permeability) has raised interest in use of probiotics in gastrointestinal disorders.

Management of GE includes fluid repletion, refeeding, zinc supplementation which has been shown to decrease stool output and duration of diarrhea.[5-7] Adsorbents (attapulgite and smectite) can potentially bind mucus, toxins and reduce water loss but only smectite was shown to reduce duration of diarrhea by one day.[8] Antibiotics and antiparasitic agents should be used when indicated.

Probiotics have been advised in the care of children with diarrhea. Although probiotics have been part of human diet in fermented food such as yogurt, kimchi etc. use of probiotics supplementation in large doses (10^{10} 10^{12} cfu) should be recognized as a deliberate attempt to alter the intestinal microflora and its potential for benefit and harm should be evaluated.

Clinically, these beneficial microorganisms are used in much larger doses than present in fermented food. Large doses, (billions of live microbes daily) are used so that at least some of the orally administered microbes may survive the gauntlet of gastric acid, proteolytic enzymes of stomach and intestine and bile. These surviving probiotic microorganisms then attach to enterocytes to produce their metabolic effects.

Modest therapeutic benefit has been noted in acute infectious diarrhea[9] including that caused by Rotavirus.[10] In undernourished children, use of ORS supplemented with Lactobacillus rhamnosus GG (10^{10} 10^{12} cfu) was associated with significant decrease in frequency and duration of diarrhea.[11]

Present information suggests that presumed infectious diarrhea be treated with probiotics preferably with Lactobacillus GG or Saccharomyces boulardii which have been most commonly studied. Probiotics also attenuate the muscle hypercontractility seen in postinfectious gut motility.[12]

Probiotics (*Lactobacillus and S. boulardii*) have shown benefit in prevention and treatment antibiotic associated diarrhea

Table 3: Preparations available in Karachi (alphabetically)

Brand (manufacture)	Supplied as	Contents	Price
Amybact (ICI Pakistan)	Sachet	Bifidobacterium BB12 90% CFU? Lactobacillus paracasei,L. casei 5% CFU? Streptococcus thermophilus, TH 4 5% CFU?	480 Rs/10
Biflor (Tabros)	Sachet	Saccharomyces boulardii 250 mg	400 Rs/10
Captain AD (PharmEvo)	Sachet Tablet	Bifidobacterium BB12 11 billion CFU/tablet	?
Enflor (Hilton)	Sachet capsule	Saccharomyces boulardii 250 mg	480 Rs/10
Enterogermina (Sanofi)	suspension	Spores of Bacillus clausii polyantibiotic resistant, 2 billion	800 Rs/20
Florabion (Promise Health Foods)	Sachet	Lactobacillus acidophilus 5 billion CFU Lactobacillus sporogenes 4 billion CFU Bifidobacterium lactis 5 billion CFU Fructo oligo saccharides 50 mg	285 Rs/5
FlorAid (Angelini)	Sachet and capsule	Saccharomyces boulardii 250 mg	
Gutcare (Searle)	Sachet and capsule	Clostridium butyricum and Bifidobacterium 420 mg	415 Rs/10
Montum (MAKSONS)	Sachet	Lactobacillus paracasei ssp paracasei and Lactobacillus acidophilus >3 billion CFU	595 Rs/10
Newflora (RG Pharmaceutica)	?	Enterococcus faecium SF68	?
Prepro (Matrix)	Sachet	Lactobacillus rhamnosus GG 5 billion CFU	400 Rs/10
Protectis (Ferozsons)	Drops and Tablets	Lactobacillus reuteri DSM 17938 1 billion CFU drops (need refrigeration)	1511.92Rs/5ml
Resiton (MAKSONS)	Sachet	Lactobacillus paracasei ssp paracasei> 1 billion CFU	595 Rs/10
Restore	Sachet	Saccharomyces boulardii 1 gram	?
Yugud	Sachet	Lactobacillus acidophilus 1 GG Bifidobacterium	?

(13).Review of 82 probiotic trials for antibiotic associated diarrhea showed 42% reduction in diarrhea. Sixty nine percent trials had used *Lactobacillus* alone or with other organisms. Fifteen trials (20%) used *S. boulardii* alone, in these the risk of antibiotic associated diarrhea was reduced 52%.[14]

Supplementation of Infant Milk Formula with Probiotics
Lactobacilli and Bifidobacterium which predominate in the stool of breast-fed infants were added to infant formula. In a meta-analysis of 11 trials of infant formula supplemented with probiotics, Lactobacilli and Bifidobacterium, it was seen that when supplemented infant formula was given at or before 28 days of life and continued for at least two weeks, the infants (n=1459) had stools which were similar in appearance and consistency to the stools passed by breast fed infants and their stool bacteria were similar to those of breastfed babies. Ten trials reported that supplemented formula was well tolerated but one reported significantly increased incidence of diarrhea, irritability and eczema in the infants receiving probiotic supplemented formula.[15] In a meta analysis of 35 trials no benefit was seen in probiotic, prebiotic or synbiotic addition to infant formula. It provided no improvement in growth or in

protection from diseases including "infantile colic" or excessive crying. No adverse effects were noted.[16] This review published in 2012 did not support routine supplementation of term infant formula with probiotics, prebiotics or synbiotics.

Thus probiotics are not recommended to treat or prevent infantile colic which generally resolves on dietary changes and parent counselling. However a meta-analysis concluded that Lactobacillus reuteri may be beneficial in reducing colic (excessive crying) in exclusively breastfed babies.[17]

Necrotizing Enterocolitis

The pathogenesis of necrotizing enterocolitis (NEC) which occurs in prematurely born neonates or small infants is multifactorial. Abnormal gut flora may play a role.

Normally in the first month of life there is an enormous shift from sterile milieu in utero to microbial colonization of gut. Normally *Lactobacilli* and Bifidobacterium predominate in infants. Antibiotic usage and the NICU environment hinder development of normal gut flora in these in infants; this may be one of the factors causing NEC.

Thus probiotics therapy seems logical for preventing NEC. A meta-analysis of trials using Lactobacillus, Bifidobacterium, *Streptococcus salivarius* and *Saccharomyces boulardii*, showed that Infants who received probiotics vs placebo were less likely to get severe NEC and had a lower mortality rate. Also there was no difference in the rate of nosocomial sepsis in the groups receiving probiotics vs placebo.[18-19] A follow up of one of the study groups showed no difference in neurodevelopment at 3 year age.[20]

Complications of Probiotics Use

The use of probiotics still remains a concern in the prematurely born vulnerable population, as systemic infections have occurred with probiotics in physically impaired hosts.Lactobacillus septicemia occurred in two children with short bowel syndrome who were receiving receiving Lactobacillus rhamnosus GG supplementation.[21]

Saccharomyces cerevisiae was recognized as an emerging infectious disease in adults when three case occurred in a two week period in an ICU. The only risk factor was treatment with probiotic containing S. boulardii (manufacturer, Bristol-Myer Squibb) for prevention of Clostridium difficile associated diarrhea. Patients had received the probiotic by nasogastric tube for mean 8.5 days before positive blood culture. Only two of four control hospitalized patients had received the probiotic. Surveillance cultures of controls were negative and S.cerevisiae with DNA identical to the blood culture isolate was cultured from the probiotic capsules.Literature review showed this fungemia to be associated with probiotic therapy in 50% patients, and with high mortality.[22]

S. cerevisiae fungemia has also been reported in an immunocompromised patient who was not treated with *S. boulardii* preparation[23] and there is a report of catheter related fungemia caused by *S. cerevisiae* in a newborn.[24] With so many patients and healthy caregivers taking probiotics as food supplement, it remains a possibility that these two cases were also related to accidental exposure to this otherwise uncommon pathogen.

The potential for contamination of probiotic preparations by other microbes with disastrous results is further illustrated by the following report: An infant developed a fatal case of intestinal mucormycosis with Rhizopus oryzae, after receiving a probiotic supplement on first four days of life. The product (Solgar ABC Dophilus powder) was supposed to contain Bifidobacterium lactis, Streptococcus thermophilus and Lactobacillus rhamnosus but the unopened samples were found to be contaminated with the fungus Rhizopus oryzae.[25]

The probiotic preparations can produce unexpected allergic results as their contents are not labelled fully. To study this risk skin tests were performed on children who were allergic to cow milk protein using three probiotic preparations. All showed an allergic response to these products (Reuterin (Lactobacillus reuteri, Bio Gaia), Fiorilac and Dicoflor). This led allergists to advise that children who are allergic to milk should not receive probiotic preparations which contain cow milk protein and that all these products should be labelled clearly, for the potential presence of cow milk protein.[26]

Children with autism often have gastrointestinal problems and diarrhea. Probiotics have been tried but no benefit nor was any harm documented.[27]

Role of Probiotics in Promoting Immunity

Although probiotics are generally promoted for use in GI disorders, they are of immense interest to allergists and pediatricians dealing with eczema and asthma.

Gut microbiota confer specific immune protective effects which are mediated through complex pathways within gut associated lymphoid tissue (GALT). This is mediated by local IgA synthesis and production of tolerogenic dendritic cells and regulatory T cells. These effects may have influence on the immune system of the whole body. The regulatory T cell population of GALT produces immunomodulatory cytokines, Interleukin 10 (IL 10) and transforming growth factor beta (TGF-beta).[28]

Atopic dermatitis, eczema, is a chronic inflammatory skin condition which generally starts in infancy.Topical corticosteroids and topical calcineurin inhibitors (tacrolimus and pimecrolimus) are the mainstay of management. Skin and nares of children with eczema are often colonized with Staphylococcus aureus In meta-analysis of trials using probiotics, the supplementation was found to decrease the incidence of eczema if it was given

to mothers during pregnancy and to babies in early infancy.[29] However a prior meta-analysis of 39 trials using probiotics on young infants had failed to show any reduction of eczema but it was found that babies receiving probiotics were more likely to spit up.[30]

The failure of probiotics in preventing eczema can be understood by following the process by which the functionally immature neonatal immune system develops a complex balance between host defences and immune tolerance and this can only occur in presence of gut flora.

Animal experiments with germ free mice show that immune function can develop if normal gut flora is restored but only in young animals. So if probiotic are given to develop a healthy gut flora and thus help develop a normal (non-allergic) response to antigenic challenge, they will need to be given very early in life.[31]

The possibility of promoting commensal bacteria and eliminating colonization of upper airway with pathogens has drawn attention in management of children who are prone to recurrent episodes of acute otitis media (AOM).

In theory administration of probiotics to a child after antibiotic treatment of AOM will re-colonizes the nasopharynx with bacteria that will hinder the growth of pathogenic bacteria and thus end nasopharyngeal colonization with the pathogens preventing recurrence.

However in two randomized trial, each of which included more than 200 children with recurrent otitis media, treatment with oral probiotics produced no change in outcome.[32,33]

Another placebo controlled trial which used the novel approach of intranasally administered spray of Alpha Streptococci kept more children AOM free for three months (42% vs 22%).[34]

Discussion

The benefit of probiotics use is outlined in Table 4. It shows that in otherwise healthy infants and children, probiotics (*Lactobacillus acidophilus*, Bifidobacterium and Saccharomyces) moderately decrease the severity and duration of acute infectious diarrhea. They are a better choice than antibiotics, smectite and nitazoxanide.

In undernourished children Lactobacillus reuterii GG decreased frequency and duration of diarrhea.
S. boulardii seems preferable in the prevention and treatment of antibiotic associated diarrhea and may be used in children receiving outpatient antibiotic therapy.

The beneficial effects may be related the type of organism being used and generalization may not be valid.

Probiotics are living organism. They cannot be sterilized and thus they carry the risk of being contaminated with pathogens and causing infections. Probiotics have the potential to cause systemic infection when mucosal or skin barriers are breached. Given the widespread use and the large numbers of living microbes in each dose we need to identify high risk groups of patients who may develop systemic disease, even if these sachets are opened in the their room, ward, ICU or NICU.

These high risk groups include all patients with central lines, all immune compromised patients; these include patients on chemotherapy with mucositis and/or neutropenia, organ transplant recepients, patients with AIDS and third degree

Table 4: Diseases in Infants and Children in Which Probiotics Have Been Used for Treatment and Prevention

Diseases	Prevention	Treatment
Gastrointestinal Diseases		
Diarrhea		
Acute infectious diarrhea (especially rotavirus)	++	++
Traveler's diarrhea	+	?
Antibiotic-associated diarrhea	++	+
Clostridium difficile	+	?
Infantile Colic	?	+
Inflammatory bowel disease		
Ulcerative colitis	?	+
Crohn disease	?	-
Helicobacter pylori gastritis	?	+
Hepatic encephalopathy	+	+
Irritable bowel syndrome	-	+
Constipation	-	-
Lactose intolerance	-	+
Pancreatitis	-	-
Allergic/Atopic Disease	+	+
Upper Respiratory Tract Infections	+	-
Neonatal Disease		
Necrotizing enterocolitis	++	-
Neonatal Sepsis	++	-
Cancer	?	?

\+ indicates limited evidence or conflicting evidence for benefit
++ indicates clear evidence for benefit.
\- Indicates no clear evidence for benefit or possible deleterious effect.
? Indicates no studies conducted to date

From Feign and Cherry's Textbook of Pediatric Infectious Diseases. 7th edition,2014.
Harrison GJ. Probiotics chapter 242, Page 3371.

malnourished young children; who frequently present with presumed infectious diarrhea.

If probiotics are used in the inpatient setting, rules should be made to open the sachets or capsules in a room away from the high risk patients and the site of intravenous fluid handling and preparation. The person mixing them in fluid or milk for in-patient oral or NG administration should wear disposable gloves and apron to not track these germs to the high risk patients.

Probiotics are stimulators of immune system; theoretically probiotics might cause excessive immune stimulation leading to autoimmune type disorders.

The risk of Gene transfer from probiotic bacteria to gut bacteria should be regarded as a risk especially when antibiotic resistant strains are used. Use of antibiotic resistant bacterial strains as probiotic, would not only prevent treatment of a rare probiotic infection but may even allow transfer of genetic material conferring resistance to other gut flora.

Strains used for probiotic therapy should be chosen from commensal human flora and bacterial strains should not carry intrinsic resistance to antibiotics. One of the preparations being given to small infants (Enterogermina drops) is in flagrant disregard of this safeguard.

These problems will be ultimately solved when biologically active isolated products of microbial agents become available and are used in probiotic therapy.

Conclusions

Therapeutic use of some probiotics, Saccharomyces boulardii and Lactobacillus species have been efficacious in decreasing the duration of infectious diarrhea and risk of antibiotic associated diarrhea. Probiotics are protective against development of NEC in prematurely born infants. There is no benefit of probiotic supplementation of normal infants.

The importance of normal gut flora in development of immunity and normal enterocyte function emphasizes the need to limit unnecessary antibiotic use in respiratory viral infections and diarrhea; Rather than correcting the subsequent deleterious effects on microflora.

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Initiation of Policy Development: "National Policy for Antimicrobial Resistance Containment"

Antibiotic resistance is one of the major health crises in Pakistan with overall situation much more grim as depicted in many published studies over last two decades. Pakistan in line with World Health Resolution in May 2015 and "One Health" approach has initiated efforts to set up a **"***National Policy for Antimicrobial Resistance Containment"* through the Ministry of National Health Services, Regulations and Coordination (MNHS&RC) after due consultative process. A national Intersectoral Core Committee (ICC) was officially established vide MNHS & RC notification in November 2015 for this specific purpose. Essential information on the country's AMR containment system was gathered and available literature was reviewed in light of WHO Global Action Plan 2015 and 2016.

The development of this policy seeks to address the challenges of AMR. Focus has been ensuring policies for key areas such as AMR awareness and education, AMR burden and surveillance, infection control and other preventive steps, antimicrobial stewardship and the use of antibiotics in other fields such as agriculture, veterinary medicine and other fields as per WHO's 5 Objectives.

The primary objective of the *"National Policy for Antimicrobial Resistance Containment"* will be to ensure that current and any new antimicrobials are effective as long as possible through concerted, comprehensive and integrated approach at national level. It will also be able to supplement the global and regional efforts on AMR involving human and veterinary medicine, agriculture, finance, research, environment and consumers.

As an assessment and gap analysis the AMR Team consisting of two consultants visited key stakeholders, public health officials, major public and private hospitals, provincial health departments, drug regulatory authority, animal and agriculture institutions and other related AMR related individuals. Specific information was sought related to awareness about AMR, hospital beds, personnel, laboratory and other facilities etc. Subsequently two consultative workshops of 2 days each and a total of 17 visits including visits to different federal and Provincial Health Care Authorities and tertiary care hospitals in different cities of the country were undertaken over last 4-5 months. During the workshops participants worked together on the development of the *National AMR Containment Policy* keeping in view five objectives of WHO Global Action Plan 2015. The first policy development workshop was held in Islamabad in February 2016 and the second workshop in April 2016. Experts from relevant fields and representatives from provinces were engaged to develop broader consensus on key issues. The workshops were conducted under the guidance of the Dr Asad Hafeez, Director General, Ministry of National Health Services Regulation and Coordination through AMR focal person Dr Muhammad Salman, Consultant Microbiologist at National Institute of Health. Dr. Ejaz Khan, infectious diseases specialist and Dr. Muhammad Usman, Consultant Microbiologist served as facilitators for the workshops. During these workshops the foundation for a national policy was built, through development of vision, performance of SWOT analysis and formulation of policy topics and statements. The visit reports were also shared with participants during the second workshop.

After the second workshop a policy dialogue process was started to discuss the draft *National AMR Containment Policy* before endorsement. This process will ensure national ownership as well as the commitment of all relevant stakeholders to the policy. The invaluable support and contribution came from the following major stakeholders: Provincial *Directorates General* of *Health*, National Agricultural Research Centre/Pakistan Agricultural Research Council, Drug Regulatory Authority of Pakistan, Pakistan Field Epidemiology and Laboratory Training Program, Ministry of Agriculture and Livestock, Pakistan Medical Research Council, **Medical Microbiology & Infectious Diseases Society of Pakistan (MMIDSP)**, *Armed Force Institute of Pathology*, Pakistan Medical Association, Pakistan Poultry Association, National Health Programs, Pakistan Nursing Council, Pakistan Pharmacy Council, Provincial *Directorates General* of Animal *Health and other representatives from other associations and the Ministry.*

As this is a first step for strategizing and then implementing AMR Containment Policy the ICC will continue to work together with a huge responsibility to monitor and oversee the whole policy process.

Instructions to Authors

Scope
The Infectious Diseases Society of Pakistan sponsors the Infectious Disease Journal of Pakistan (IDJ). The Journal accepts Original Articles, Review Articles, Brief Reports, Case Reports, Short Communications, Letter to the Editor and Notes and News in the fields of microbiology, infectious diseases, public health; with laboratory, clinical, or epidemiological aspects.

Criteria for publication
All articles are peer reviewed by the IDSP panel of reviewers. After that the article is submitted to the Editorial Board. Authors may submit names and contact information of 2 persons who potentially could serve as unbiased and expert reviewers for their manuscript, but IDSP reserves the right of final selection.

Submission of the Manuscript
Manuscripts must be formatted according to submission guidelines given below, which are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (originally published in *N Engl J Med* 1997;336:309-15). The complete document appears at www.icmje.org. Please submit one complete copy of the manuscript and all enclosures to **The Managing Editors, Infectious Diseases Journal of Pakistan, Department of Pediatrics & Child Health, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan.** An electronic copy of the manuscript must also be sent to pak_idj@yahoo.com**.** All manuscripts submitted to IDJP must be accompanied by an Authorship Declaration stating that *'The authors confirm that the manuscript, the title of which is given, is original and has not been submitted elsewhere.Each author acknowledges that he/she has contributed in a substantial way to the work described in the manuscript and its preparation'*. Upon submission a manuscript number will be assigned which should be used for all correspondence.

Manuscript Categories

I. Original Articles
Articles should report original work in the fields of microbiology, infectious disease or public health.The word limit for original articles is 2000.

Title page
This should list the (i) title of the article, (ii) the full names of each author with highest academic degree(s), institutional addresses and email addresses of all authors. (iii) The corresponding author should also be indicated with his/her name, address, telephone, fax number and e-mail address. (iv) A short running title of not more than 40 characters (count letters and spaces) placed at the foot end of the title page.(v) a conflict of interest statement should also be included in this section.

Abstract
Abstract should not exceed 250 words and must be structured in to separate sections headed *Background, Methods, Results and Conclusions*.

Please do not use abbreviations or cite references in the abstract. A short list of four to five key words should be provided to facilitate.

Background
The section must clearly state the background to the research and its aims. Controversies in the field should be mentioned. The key aspects of the literature should be reviewed focusing on why the study was necessary and what additional contribution will it make to the already existing knowledge in that field of study. The section should end with a very brief statement of the aims of the article.

Materials and Methods
Please provide details of subject selection (patients or experimental animals). Details must be sufficient to allow other workers to reproduce the results. The design of study and details of interventions used must be clearly described. Identify precisely all drugs and chemicals used, including generic name(s) and route(s) of administration All research carried out on humans must be in compliance with the *Helsinki Declaration*, and animal studies must follow internationally recognized guidelines. The authors are expected to include a statement to this effect in the Methods section of the manuscript. A description of the sample size calculation and statistical analysis used should be provided.

Results
Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of 5 illustrations (in a combination of figures and tables) per article. The results should be in past tense and repetition of results presented in the tables should be avoided. Exact *P*-values should be reported along with reporting of OR and RR with their Confidence Intervals where applicable.

Discussion
Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat the details from the results section. Discuss the implications of the findings and the strengths and limitations of the study. Link the conclusions with the goals of the study but avoid unqualified statements and conclusion not completely supported by your data.

Acknowledgments
Acknowledge any sources of support, in the form of grants, equipment or technical assistance. The source of funding (if any) for the study should be stated in this section. Please see below for format of **References, Figures and Tables**.

II. Review Articles
Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. It should consist of critical overview of existing literature along with reference to new developments in that field. These should be comprehensive and fully referenced. Articles should contain an Abstract; Main Text divided into sections, Conclusions and References.

III. Brief Reports
Short clinical and laboratory observations are included as Brief Reports. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references.

IV. Case Reports
Instructive cases with a message are published as case reports. Routine syndromes or rare entities without unusual or new features are invariably rejected. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references. The authorship should not exceed 3-4 persons.

V. Letter to the Editor
These may relate to material published in the IDJP, topic of interest pertaining to infectious diseases, and/or unusual clinical observations. A letter should not be more than 300 words, one figure and 3-5 references.

VI. News and Views
Informative, breaking news updates in infectious diseases from around the world (approx. 200 words).

VII. Notices
Announcements of conferences, symposia or meetings may be sent for publication at least 12 weeks in advance of the meeting date. Details of programs should not be included.

References
Number references consecutively in the order in which they are first mentioned in the text. Identify references in text, tables and legends by Arabic numerals (in superscript). References cited only in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification of the particular table or illustration. Bibliography should be given in order. Authors, complete title, journal name (Abbr), year, vol, issue, page numbers. According to "Uniform Requirements of Manuscripts submitted to Biomedical Journals", as cited in N Engl J Med 1997; 336:309-15.

Tables and Figures
Data reported either in a table or in a figure should be illustrative of information reported in the text, but should not be redundant with the text. Each table must be presented on a separate sheet of paper and numbered in order of appearance in the text. Table should be numbered consecutively in Arabic numerals. Tables and Figures legends should be self-explanatory with adequate headings and footnotes.Results which can be described as short statements within the text should not be presented as figures or tables.

Illustrations
Illustrations should be numbered, given suitable legends and marked lightly on the back with the author's name and the top edge indicated. Original drawings may be submitted although high quality glossy photographs are preferable. They should be kept separate from the text. If possible, figures should be submitted in electronic format as either a TIFF (tagged image file format) or JPEG format. Minimum resolution for scanned artwork is:

- Black& white line illustration (e.g. graphs): 600 dpi
- Black & white halftone illustrations (e.g. photographs): 300 dpi
- Color illustrations: 400 dpi (note that color images should be split CMYK not RGB)

Plagiarism
Authors should refrain from plagiarism and should double check their work before submitting it for publication. Adequate references should be provided for text from other sources.

Authorship criteria
Those who have contributed sufficiently to the conceptualization, design, collection and analysis of data and writing of the manuscript should be granted authorship. Ideally all authors should be from the same department except for studies that are multi center or multispecialty.

Instructions updated - April 2012.
Editor IDJ