A STUDY OF INFECTIONS AND INFESTATIONS IN PROTEIN ENERGY MALNOURISHED CHILDREN IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

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M.D. (MICROBIOLOGY)

BRANCH – IV

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CHENNAI, INDIA

CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON THE PREVALANCE OF INFECTIONS AND INFESTATIONS IN PROTEIN ENERGY MALNOURISHED CHILDREN IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR. K.M.PAVENDAN,** during the period of his Post graduate study from June 2008 to April 2010 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2010.

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DECLARATION

I declare that the dissertation entitled **"A STUDY ON THE PREVALANCE OF INFECTIONS AND INFESTATIONS IN PROTEIN ENERGY MALNOURISHED BABIES IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **May 2009 to May 2010** under the guidance of Professor **Dr. N. DEVESENA M.D.,** Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Microbiology (Branch IV) examination to be held in April 2010.

Place: Chennai
Date :

Signature of the Candidate
(Dr.K.M.PAVENDAN)

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CONTENTS

INTRODUCTION

PEM is a spectrum of conditions caused by varying levels of protein and calorie deficiencies.[44]

Primary PEM is caused by social or economic factors that result in a lack of food. [26].

Secondary PEM[26] occurs in children with various conditions associated with increased caloric requirements (infection, trauma, cancer), increased caloric loss (malabsorption), reduced caloric intake (anorexia, cancer, oral intake restriction, social factors), or a combination of these three variables.[44]

Classification Guidelines for Pediatric Undernutrition[27]

Nutrition Status	Weight/Age	Height/Age	Weight/Height	% IBW
Wasting	Normal or low	Normal	<5th percentile	<85-90%
Stunting	<5th percentile	<5th percentile	Normal	Normal
Mild malnutrition	Normal or low	Normal	<5th percentile	81-90%
Moderate malnutrition	Normal or low	Normal	<5th percentile	70-80%
Kwashiorkor	Normal or low	Normal or low	Normal (edema)	Normal
Marasmus (severe wasting)	Low	Normal or low	<5th percentile	<70%

IAP grading of malnutrition[16]

*Nutritional Status**	*Weight for age (% of expected)*
Normal	> 80
Grade 1	71-80
Grade 2	61-70
Grade 3	51-60
Grade 4	< 50

* If the child has edema of nutritional origin the letter K is placed along with the grade of PEM to denote kwashiorkor.

COMPLICATIONS OF MALNUTRITION

Malnourished children are more susceptible to **infection**, especially sepsis, pneumonia, and gastroenteritis. **Hypoglycemia** is common after periods of severe fasting, but also may be a sign of sepsis. **Hypothermia** may signify infection or, with bradycardia, may signify a decreased metabolic rate to conserve energy. Bradycardia and poor cardiac output predispose the malnourished child to heart failure, which is exacerbated by acute fluid or solute loads[44]

Micronutrient deficiencies also can complicate malnutrition[44]. Vitamin A and zinc deficiencies are common in the developing world and are an important cause of altered immune response and increased morbidity

and mortality. Depending on the age at onset and the duration of the malnutrition, malnourished children may have permanent growth stunting (from malnutrition in utero, infancy, or adolescence) and delayed development (from malnutrition in infancy or adolescence). Environmental (social) deprivation may interact with the effects of the malnutrition to impair further development and cognitive function.

AIMS AND OBJECTIVES

- Analysis of infections (bacterial) and infestations (Parasitic) in babies attending outpatient and inpatient department of Institute of Child health with the symptoms of Protein Energy Malnutrition.
- Correlating symptoms associated with Protein Energy Malnutrition and Microbial infections and Infestations.
- Comparison of incidence of infections with general pediatric population attending Institute of Child Health for other diseases.

REVIEW OF LITERATURE

Protein-Energy Malnutrition

DEFINITION

Protein-energy malnutrition has been considered a range of pathological conditions arising from a coincident lack of varying proportion of proteins and calories. Occurring most frequently in infants and young children, and commonly associate with infection[44].

The most obvious feature of PEM is reduction or failure of growth[45]. Different organ systems are altered to varying degrees by PEM, with tissues, which have the highest rates of turnover being most severely affected. Two types of tissue expedient especially high rates of turnover, and therefore are particularly susceptible to deprivation of essential nutrients. First, tissues with a high-rate of all turnover, such as intestine, testes, and bone marrow. The other type of tissue is susceptible to an insufficiency of essential nutrient contents, liver and pancreas, which are metabolically very active and experience a high rate of cytoplasmic turnover[30].

Two major categories of PEM are kwashiorkor and marasmus[32,7]. Kwashiorkor and marasmus are different events during homologous dietary deficiencies. The symptomology of marasmus is thought to be the result of the extreme degree of adaptation of the body to a deficient diet. In this situation, essential organs and function are protected at the expense of fat

and muscle tissue. Kwashiorkor results after the host can no longer adapt to the nutritional stress.

Nearly all aspects of homeostasis in the malnourished organism are affected by metabolic and biochemical abnormalities, such as altered metabolism of proteins and amino acids. Perhaps the best indication of altered protein metabolism in PEM is a reduction in the level of circulatory plasma protein, the albumin fraction being particularly affected [11].

It is known that the polysomal profile is markedly affected by nutritional condition, which would certainly have a major effect on the rate of protein synthesis and degradation [11].

Host Immune Response in Protein-Energy Malnutrition

The immune system is enormously complex and highly integrated, with "built-in" checks and balances to regulate each antigen-specific response. This complexity reflects a system that is rapidly responsive, continually vigilant, highly precise and sensitive in terms of antigen recognition and detection, capable of amplification, and possessed of a superb capacity for memory [15]. However, this complexity also permits a variety of extraneous factors to influence or impair immune system functions. Nutritional status and intercurrent infections are among the most important of the extrinsic influencing factors [34,15].

The resistance of the host against bacterial infection is dependent on lymphocytes, antigen specific, that participate in the humoral, the beta-cell system of antibody production, and the T-cell system of cell mediated-immunity.

These systems work in conjunction with several nonspecific factors of resistance, such as, skin, mucous membranes, complement, phagocytic cells, lysozyme, and bactericidal capacity of blood and tissue phagocyte [15,34]. T-lymphocytes, predetermined clone proliferate, produce molecules responsible for increasing the phagocytic and bactericidal ability of monocytic blood cells and also directly participate in graft rejection, cytotoxicity against cancer cells, delayed-type Hypersensitivity, suppression of the autoimmune phenomenon, and regulation of the immune response [15].

Malnutrition, Infections, and Immunity

Most attention during the period of immune nutritional reemergence focused on effects of PEM on the immune system and other host defensive mechanism. The myriad observations, some quite old and some quite new clearly show that the immune system cannot function optimally if malnutrition is present. Malnutrition also produces adverse effects on antigenically nonspecific mechanism of host defense. Careful observation

showed a correlation between nutritional status and morbidity and mortality largely due to infections.

The initial work on interaction between nutrition and immunity was carried out in young children with PEM [15,34]. It was seen that infection and malnutrition were invariably linked together, each aggravating the other. It can be caused by several factors, such as lack of health education, illiteracy, contaminated food and water, poor sanitation and overcrowding are important in worsening the situation. The hypothesis that depressed immune system in malnutrition enhances the risk and severity of infections are compatible with the consistent impairment of immunity in PEM and hospitalized patient with primary immuno-deficiencies[19].

The Effect of Protein Energy Malnutrition on the Immune System

Malnutrition can cause dysfunction of various organ systems. The degree of severity on these organs depends on several factors, including the amount and rate of protein synthesis, the rate of cell proliferation, and the role of individual Nutrients in metabolic pathway. Lymphoid tissues are very vulnerable to these damaging effects.

Many cells of the immune system are known to depend for their function on metabolic pathway that employ various nutrients as critical factors. The most consistent changes in immune competence in PEM are in cell-mediated immunity, the bactericidal function of neutrophils, the

complement system and the secretory immunoglobulin A (IgA) antibody response [36,11,34].

According to modern definitions, "Cell-mediated immunity is immunity whose induction and expression is T-cell dependent. " Cell-mediated immune is regarded as non humoral immunity having to do with delayed-type hypersensitivity in the skin test and T-cell functions . Many of these parameters have been used in malnourished host studies, including the anatomy of lymphoid organs, allograft rejection, transfer of delayed hypersensitivity, in vitro test of T-cell function and T-cell dependent antibody synthesis[15].

Depression of cell mediated immunity by PEM could be the result of an absolute or relative deficiency of amino acids for cell multiplication. Alternatively, children with PEM are known to have raised levels of plasma cortisol, which can decrease the thymolymphatic system and repeated infection may also play a part[18]. Decreased T-cell numbers in protein-malnourished animals is caused by decreased synthesis of thymic hormones. Thymic hormones are responsible for the development of the cell-mediated immune response[12].

Clinical and histopathological studies have shown that the most consistent changes in immuno-competence are impaired cell mediated immunity and humoral immunity moderate or severely[1]. The lymphoid

tissues, particularly the thymus were found to be atrophied, reduction in delayed cutaneous hypersensitivity, fewer T-cells, decreased thymulin activity[9].

Impaired secretory immunoglobulin A antibody response and reduced concentration and activity of complement components. The mechanism by which the T-lymphocyte population is gradually depleted from the thymus or lymphoid organs during malnutrition is not readily apparent[7].

The effect of several nutrients, particularly trace elements and lipids on the afferent and effector limbs of the immune response should be studied, because a number of observations indicate the crucial role of several nutrient in key metabolic pathways and cell functions, that frequently complicate PEM and many systemic diseases. Moreover, human malnutrition is usually a complete syndrome of multiple nutrient deficiencies, in addition, PEM children have clinical, biochemical, and hematological changes (e.g., weight loss, muscular atrophy, enlarged liver, loss of subcutaneous tissue, and edema) depending on the degree of malnutrition[6].

The analysis of the molecular basis of immunodeficiency in PEM is in its infancy, also is needed for future research. The influence of nutrition on antigen-non-specific mechanism of host resistance has also been

inadequately studies and the biological relevancies of impaired immunity on malnutrition.

Position in India

In any community mothers and children constitute a priority group. In India women of the child bearing age group (15-44) years constitute 40 % and children under 15 years of age about 19 % of the total population [31]. Together they contribute to nearly 59 % of the total population of the country. Mothers and children not only form a large group, but they are also a vulnerable or special risk group. The risk is connected with child bearing in the case of women; and growth and development and survival in case of infants and children. In developing countries 50 % of all deaths are occurring among children less than 5 years of age.

Maternal and child health (MCH) services were started in the early 1950s with the aim of improving the health status of mother and children. To start with the programme concentrated on the antenatal, intra natal, and post natal care and to enhance mother and child survival. In 1992, the programme was re launched as the Child survival and safe motherhood programme with emphasis being on regular antenatal, intranatal, postnatal care, immunization, family welfare activities, nutritional services, promotion of breast feeding and proper health education, which has brought about drastic changes in the health indicators pertaining to mother and child.

Proper antenatal care including tetanus toxoid immunization, iron and folic acid supplementation combined with good intranatal and postnatal care followed by the early introduction of breast feeding can reduce the mortality and morbidity of children. But still neonatal deaths contribute to more than fifty per cent of infant deaths raising concern over the MCH services.

Though the incidence and mortality due to ARI and AWD diseases have come down reasonably in recent years, they contribute significantly to morbidity of the children.

Regarding the nutrition aspect both ICDS and TINP services are aimed to improve the nutrition of children. But the nutritional status of children needs considerable improvement[28]. Tamilnadu enjoys a special place above the national average in many of the socioeconomic and health indicators. Various studies done at the state level showed a better prevalence of child health indicators in urban areas than rural areas[41]. Poor health indicators in rural areas have been attributed to certain factors like poverty, lack of education, overcrowding, lack of safe water and basic sanitation, and finally poor health facilities. Although the Government has taken lots of steps to improve health facilities in rural areas, lack of education and various social and cultural factors have contributed to the poor health seeking behavior in rural areas which in turn may be responsible for the poor child health indicators.

Urbanization and its consequences (43)

Living conditions have a direct impact on public health. Just 26% of the population in India lives in urban areas, but the increase in the number of people living in urban areas has risen remarkably. One of the biggest challenges that face urban planners worldwide is the proliferation of slums in urban areas and the host of health hazards that they bring along. Maharashtra and Gujarat are the most urbanized states in the country. Urbanization has led to increased productivity and economic diversification, but also deprivation, poverty, and marginalization. Of the country's total population 20-25% live in slums, often located near factories, power stations, garbage dumps, and busy roads. The increased demand for services and infrastructure has depleted natural resources and caused other environmental problems.

Environmental problems include those caused by widespread poverty and those caused by industrialization and a change in consumption patterns. The cumulative impact of these 2 causes has serious effects on urban dwellers, especially slum people. Low incomes, illiteracy, and inaccessibility to development opportunities further complicate problems[5]. Slum dwellings have no ventilation or natural light and are vulnerable to fire. Slum dwellers suffer from dust, smoke, and noise pollution. Piles of garbage, potholes, stray animals, flies, and mosquitoes are common.

Urbanization has increased disease-producing agent, e.g., toxic chemicals and car exhaust fumes[10]. Lead emitted from car exhausts or industry causes reduced fine motor coordination, hyperactivity, lower IQs, and perceptual problems in slum children. Few slums have access to potable water and sanitation services. Children in slums are more vulnerable to diseases and deficiencies than their rural and other urban counterparts. Many people in slums are rural migrants. Urban pressures weaken traditional family ties and social control over children. Adult supervision of children is rare. Schools are not always available. Interaction between children and parents, recreation, and cultural stimulation are all lacking. Child labor is also common[21].

Nutritional Status

IAP grading of malnutrition (1972)[16]

Nutritional Status*	***Weight for age (% of expected)***
Normal	> 80
Grade 1	71-80
Grade 2	61-70
Grade 3	51-60
Grade 4	< 50

* If the child has edema of nutritional origin the letter K is placed along with the grade of PEM to denote kwashiorkor.

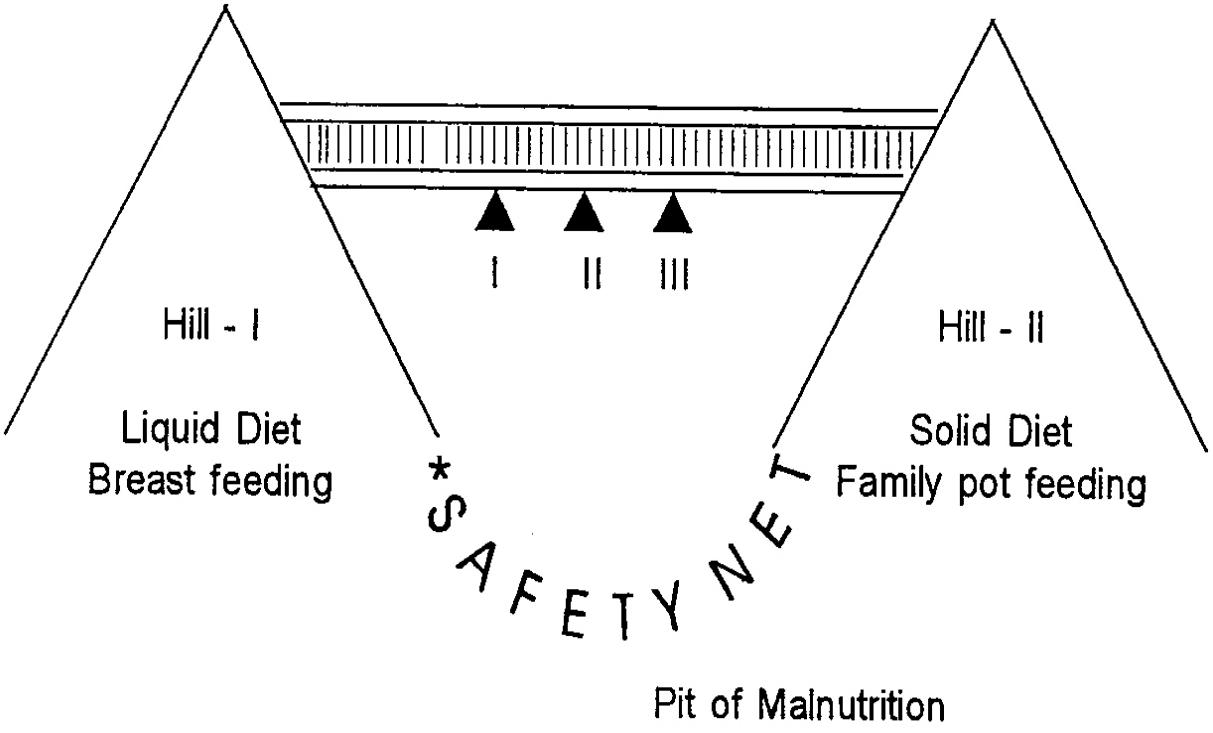
Applicable to children in the age group of 1-5 year

"Malnutrition is often found to start in the womb and end in the tomb" [37].

Severe forms of malnutrition like marasmus and Kwashiorkor represent only a tip of the iceberg[37]. Many more suffer from moderate, mild (or) invisible PEM malnutrition increases morbidity and mortality. Due to various cultural influences like food habits, customs, beliefs, traditions, religion, food fads to cooking practices, child rearing practices, attitudes and superstitions, people tend to consume poor diet when good ones are

available. Thus lack of food is not the only problem. Often there is starvation in the midst of plenty[32].

Premature curtailment of breast feeding, adoption of bottle feeding and change over from locally available food to commercially prepared refined foods are some of the child rearing practices that adversely affect the nutritional status of children[39]traditional hand feeding should not be looked down upon. This is a traditional method of eating throughout India. What should be stressed is that the hand should be clean, and the nails cut short and scrubbed frequently.



Weaning or complementary Bridge & Safety Net to Prevent PEM

I – Continued breast feeding

II - Vegetables Protein

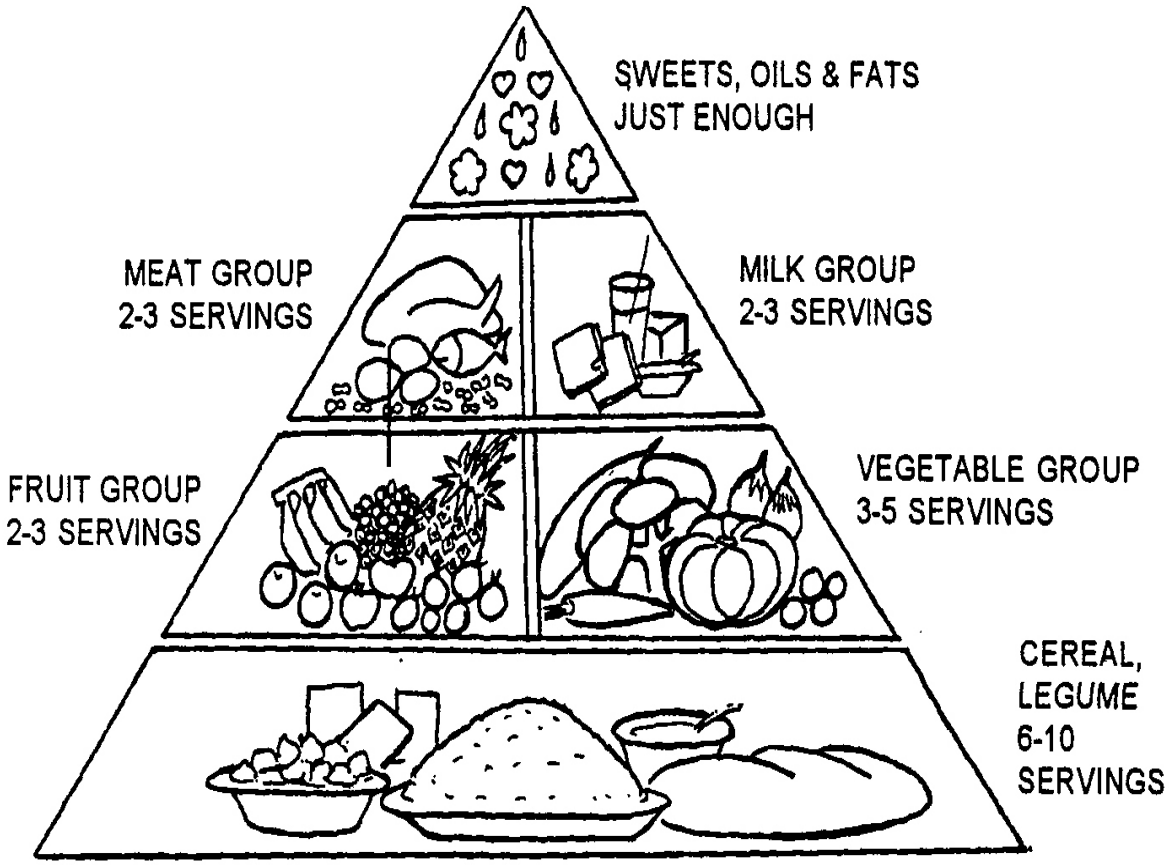
III - Animal Protein

- Supplementary feeding

- Group Eating

- Small frequent Feed.

Complementary Bridge and Safety net to prevent PEM.



Food guide triangle for day to day choice of food.

MATERIALS AND METHODS

Place of study

Institute of Microbiology

Madras Medical College,

Chennai-3

And Institute of Child Health and Hospital for Children

Egmore, Chennai-8

Period of Study

Prospective study carried out over a period of one year from June 2009 - May 2010.

Study Design

Descriptive study.

Sample Specification

Study population: 100 children less than age of five months with symptoms of bacterial or parasitic infection and infestation and falling into criteria of Indian Association of Pediatrics classification of Protein Energy Malnutrition were included in the prospective study. A detailed prenatal and postnatal history, socioeconomic histoty, history of weaning practices with special emphasis on any predisposing factors for infection and thorough clinical assessment were carried out.

Inclusion criteria:

- PEM – IAP classification
- Age of less than five years
- Suspicion of bacterial or parasitic disease
- Attending OP or admitted IP in ICH

Exclusion criteria

- Babies with weight more than standard
- PEM babies without suspicion of any disease

Methodology

RISK FACTOR PROFORMA

- -name
- Age
- Sex
- Address
- Education
- Religion
- Symptoms of PEM
- Socioeconomic status
- Referring centre

PREDISPOSING FACTORS OF PEM –PROFORMA

- Bottle feeding
- Maternal malnutrition
- LBW
- Prematurity
- Twins
- Malformations
- Artificial feeding
- Prolonged breast feeding
- Early weaning
- Food fads
- Infections
- Immunization status
- Poor diet
- Poor spacing
- Working parents
- Pregnancy in mother
- Living conditions
- Ignorance poverty

CLINICAL HISTORY PROFORMA

- H/O PEM
- Birth history if relevant
- H/o focal infections or infestations
- H/o associated risk factors
- H/o recurrent infections if any

TO EXAMINE FOR COMMON INFECTIONS

- Pneumonia
- Otitis media
- UTI
- Diarhoea
- Skin infections
- Parasitic infections

Sample Collection:

BLOOD:

The skin over the Venipuncture site is cleaned with 70% alcohol and 2% tincture of iodine. About 0.5-1ml of blood was collected and directly inoculated into the Brain heart infusion broth and Robertson cooked meat broth with liquid paraffin overlay under aseptic precautions and incubated at 37 degree centigrade for 24hours.

Sample processing

After 24hrs of incubation, the cultures were examined for turbidity, lysis of red blood cells or clotting. From Brain heart infusion broth, a Gram smear was made and subcultures were done onto Macconkey Agar, Blood Agar, Chocolate agar and Nutrient Agar and incubated at 37˚C for 24hrs. Blood agar and Chocholate agar plates were incubated at 37˚C in CO2 environment (Candle jar).S/C were done onto two Sabouraud's Dextrose Agar slant, one incubated at 37˚C and the other at 22˚C.

From Robertson cooked meat broth subculture was done onto 5% sheep blood agar with Kanamycin, Hemin and Vitamin K and was incubated under anaerobic environment for 72hours at 37˚ using polycarbonate jar with Gaspak. All bottles were reincubated and checked for turbidity daily and subcultured whenever there were any signs of growth. A final subculture was made on the tenth day on to the above media[29].

URINE:

Periurethral or perianal area is cleaned with two to three gauze pads saturated with disinfectant using a forward to back motion, followed by a wipe with sterile water or saline.

Midstream portion of the urine is then collected in a sterile wide mouthed container that can be covered with a thightly fitting lid.

Specimens are then plated in CLED media, Maconkey agar and nutrient agar and 55 sheep blood agar and studied for growth after 24 and 48 hours.[24]

SPUTUM:

As far as possible, early morning sputum samples are collected. In some babies, nebulised saline is used to induce sputum. Quality of sputum is graded with Bartlett's grading method.

The collected sample is processed as soon as possible. Gram's staining is done and examined under oil immersion microscope. Then the specimen is inoculated into chocolate agar (aerobic and with 5% co2), blood agar, differential agar like maconkey agar. The growth if any is examined after 24 and 48 hours[22,3].

ENDOTRACHEAL ASPIRATE:

The sputum sample or the endotracheal aspirate is collected through a catheter introduced in larynx into trachea. Or via endotracheal tube if in situ. Then the sample is processed as above as for the sputum[23,4].

STOOL:

Stool sample is collected with a freshly voided specimen in a wide mouthed container with tightly fitting lid.

Stool examination is performed mainly by two methods. A direct wet mount with or without iodine and by concentration method.

A direct wet mount is prepared with a slide with appropriate amount of the sample and then viewed under the microscope for evidence of parasitic forms.

Then a drop of iodine is added to another freshly prepared slide and again viewed under the microscope for evidence of parasitic forms.

A test tube is then centrifuged with sample and formalin (concentration method) to separate fecal material from parasites. Sediment is then examined for evidence of parasitic forms.

Examination of subcultures

The plates were examined after incubating for 18-24hrs. When any growth was seen, the colony morphology was noted. The colonies were identified by

1. Gram stain- to identify gram positive and gram negative organisms.
2. Hanging drop- to find out motile and non motile organisms.
3. Preliminary tests like Oxidase, Catalase were performed.

4. Members of the species were identified based on biochemical parameters using IMViC reaction and sugar fermentation medium.

COLONIAL MORPHOLOGY

Klebsiella pneumoniae

Nutrient agar

Large, dome shaped mucoid colonies.

Blood agar

Large, grayish white, mucoid colonies.

Mac Conkey agar

Lactose fermenting mucoid colonies.

Species were identified with biochemical reactions

BIOCHEMICAL REACTIONS

Indole Negative

Methyl red test Negative

Voges Proskauer positive

Citrate Utilised

Glucose Acid with gas

Lactose Acid with gas

Sucrose Acid with gas

Maltose Acid with gas

Mannitol Acid with gas

Triple sugar iron Acid / Acid with gas no H2S

Urease Positive

Escherichia coli:

Nutrient agar:

Colonies were 1-3 mm diameter, circular, low convex, smooth colonies with no pigmentation and odour.

Blood agar:

Greyish white colonies with haemolysis.

Mac Conkey agar:

Flat, lactose fermenting colonies.

Species were identified with biochemical reaction.

BIOCHEMICAL REACTIONS

Indole Positive

Methyl red test Positive

Voges Proskauer Negative

Citrate Not utilised

Glucose Acid with gas

Lactose Acid with gas

Sucrose Acid with gas

Maltose Acid with gas

Mannitol Acid with gas

Triple sugar iron Acid / Acid with gas no H_2S

Urease Negative

Pseudomonas aeroginosa

Nutrient agar:

Colonies were large, low convex with serrated margins, with bluish green pigmentation and earthy odour.

Blood agar:

Diffuse hemolysis present.

Mac Conkey agar:

Non lactose fermenting colonies with pigmentation.

Species were identified by biochemical reactions

Indole Negative

Methyl red test Negative

Voges Proskauer Negative

Citrate Utilised

Glucose Oxidatively utilised

Lactose Oxidatively utilised

Mannitol Not fermented

Xylose Oxidatively utilised

Triple sugar iron Alkali/ no change in the butt

O-F Test Oxidatively utilised

Urease Positive

Acinetobacter species:

Blood agar:

Smooth, opaque, raised, creamy colonies.

Mac Conkey agar

Non lactose fermenting colonies.

Indole Negative

Citrate Utilised

Glucose Not fermented

Triple sugar iron Alkali/ no change in the butt

O-F Dextrose Positive

Urease Negative

Staphylococcus aureus:

Nutrient agar:

Showed 1 to 3 mm diameter, circular, smooth, low convex, glistening, densely opaque colonies with golden yellow pigmentation.

Blood agar:

Colonies were surrounded by a narrow zone of beta hemolysis.

Mac Conkey agar:

Colonies were pink and small in size.

Slide Coagulase Test:positive

Tube coagulase test:positive

BIOCHEMICAL REACTIONS

Indole Negative

Methyl red Positive

Voges Proskauer Positive

Mannitol Fermented

Urease Positive

Group B Streptococci (Streptococcus agalactiae)

Blood agar:

Colonies are 1-2mm diameter, grey, mucoid with β type of hemolysis

CAMP Test:

A sterile blood agar plate was taken. A single streak of Staphylococcus aureus (β hemolysin producing Staphylococcus aureus) was made in the centre of the plate. A steak of the β hemolytic Streptococcus was made perpendicular to the Staphylococcal streak, such that the two organisms were not touching each other. Known group A and Group B Streptococcal strains were inoculated in the same plate as negative and positive controls, respectively. The plate was incubated at 35°C in ambient air for 18-24hours. An area of increased hemolysis occurs where the β

hemolysin secreted by Staphylococcus and the CAMP factor secreted by the Group B Streptococcus intersect.

Hippurate hydrolysis: To 0.4ml of 1% solution of sodium hippurate, a loopful of growth from blood agar was added and incubated at 37° C for two hours. After incubation 0.2ml of ninhydrin solution was added and incubated at 37°C for 10 minutes. The medium changes into purple colour due to hippurate hydrolysis.

Susceptibility to Bacitracin : Resistant.

Antimicrobial susceptibility tests:

Antimicrobial susceptibility of the isolates were tested using Kirby Bauer disc diffusion technique as per CLSI guidelines16. Medium used: MHA (Muller Hinton Blood agar plate was used for GBS isolate)

Inoculum: 0.5 McFarland turbidity, lawn Culture.

Incubation: 37˚C ambient air, incubated for 16-18 hrs

Preparation of inoculums:

About 4-5 colonies of the same morphology were picked up with straight wire and inoculated in 5ml of peptone water, incubated at 37°C for 3-5 hrs to attain 0.5 Mc Farland's turbidity.

A sterile cotton swab was dipped into it and pressed firmly against the wall of the test tube to remove excess broth from the swab. Dried surface of Mueller Hinton Agar plate was swabbed in three directions approximately

60° each time to ensure an even and complete distribution of the inoculum over the entire plate.

The anti microbial discs were dispensed on the agar plate an pressed down to ensure complete contact with agar surface. Disc were distributed evenly so that they were not closer than 24mm from centre to centre. Not more than 6 discs were placed in the plate.

After 16-18 hrs of incubation each plate was examined. The diameter of the zones of complete inhibition was measured including the diameter of the discs. Zones were measured to the nearest whole 'mm' using a ruler which was held on the back of the inverted petri plate.

Control strains used were

1. *Staphylococcus aureus ATCC 25923*
2. *Escherichia coli ATCC25922*
3. *Pseudomonas aeroginosa ATCC 29823*

Screening of ESBL producing strains for *Klebsiella pneumoniae*

Clinical and Laboratory Standards Institute has developed screening test for identifying the ESBL producing *Klebsiella* species.

According to CLSI guidelines, strains showing zones of inhibition of ≤ 22mm for ceftazidine, ≤ 27mm for cefotaxime were selected for conformational tests of ESBL. *Klebsiella pneumoniae* ATCC 700603 ESBL

positive strain was used as control along with *Escherichia coli* ATCC 25922 as negative control.

ESBL confirmatory test:

1. Double Disc Synergy test (DDST)

The isolated colonies were inoculated in peptone water at 37˚C for 2-6 hrs. The turbidity was adjusted to 0.5 Mc Farlands standard and lawn culture was made on Muller Hinton agar using sterile swab.

Augmentin disc (20/10 μg) was placed in the centre of plate. Both side of Augmentin disc, a disc of cefotaxime (30 μg) and cetazidime (30 μg) were placed with centre to centre distance of 15mm to centrally placed disc. The plate was incubated at 37˚C overnight, ESBL production was interpreted as the 3rd generation Cephalosporin disc inhibition wasincreased towards the Augmentin disc or if neither discs were inhibitory alone but bacterial growth was inhibited where the two antibiotics were diffused together.

2. Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL

Antibiotic susceptibility testing was done on Muller Hinton Agar with 0.5 Mc Farlands standards of the organism. Lawn Culture of the organism was made and 3rd generation Cephalosporins Cefotaxime (30μg) disc and Cefotaxime with Clavulinic acid (30 μg + 10 μg) disc was placed with

25mm apart. An increase of ≥ 5mm in zone of inhibition for Cefotaxime with Clavulinic acid compared to Cefotaxime was confirmed as ESBL producers.

RESULTS

TABLE-1

SEX DISTRIBUTION OF PEM (n=100)

55% of male babies under five years and 45% of female babies under five years are affected with protein energy malnutrition.

Sex	Percentage
Male	55%
Female	45%

TABLE-2

AGE DISTRIBUTION OF PEM (n=100)

22% of the babies under six months of age are malnourished and 78% of the babies from age 6months to 5 years are malnourished. Of the 100 babies, 44%, 30%, and 26% were suffering from grade I, grade II and grade III malnutrition by IAP standards respectively.

Age	Percentage
0-6months	22%
6months-5years	78%

TABLE-3

COMMON INFECTIONS IN PEM (n=100)

The severely malnourished children showed a very high incidence of infection (74% vs. 44% in normal babies studied), particularly diarrhoea, respiratory tract infections (RTI), bacterial skin infection, and urinary tract infections (UTI).

TABLE-4

PNEMONIA IN PEM (n=100)

Of the babies with PEM and pneumonia (40 babies), Klebsiella pnemoniae accounted for 25 cases (62.5%), staphylococcus aureus accounted for 14 cases (35%) and Pseudomonas for 1 case (2.5%).

Organism	Cases	Percentage
Klebsiella	25	62.5
Staphylococcus	14	355
Pseudomonas	1	2.5

TABLE-5

GASTEROENTERITIS IN PEM (n=100)

Of total 18 babies with PEM and gasteroenteritis, E.coli caused 12 infections (66.6%), Solmonnela 4 cases (22.2%), Klebsiella 2 cases(11.1%).

Organism	Cases	Percentage
E.coli	12	66.6
Salmonella	4	22.2%
Klebsiella	2	11.1%

TABLE-6

UTI IN PEM (n=100)

Of 22 cases of urinary tract infection, E.coli caused 16 (72.7%), Klebsiella 4 (18.1%) and Proteus 2(9.09%) were other organisms to cause infections.

Organisms	Cases	Percentage
E.coli	16	72.7
Klebsiella	4	18.1
Proteus	2	9.19

TABLE -7

SKIN INFECTIONS IN PEM (n=100)

Of 28 cases of skin infections, Staphylococus aureus caused 16 (57.14%), Pseudomonas caused 6 (21.4%), and Escherichia coli caused 2(14.2%).

Organism	Cases	Percentage
Staphylococcus	16	57
Pseudomonas	6	21.4
E.coli	2	14.2

TABLE-8

SEPTICEMIA IN PEM (n=100)

Total of 2 cases (2%) of septicemia, one each was caused by Klebsiella and staphylococcus aureus .

Organism	Cases	Percentage
Staphylococcus aureus	1	50
Klebsiella	1	50

TABLE-9

PARASITIC INFECTIONS IN PEM (n=100)

Of the total of 4 cases of stool with parasites, three were of Ascaris lumbricoides and one with Strongyloides stercoralis.

Parasite	Cases	Percentage
Ascaris lumbriciodes	3	75
Strongyloides	1	25

TABLE-10

COMMON INFECTIONS IN CONTROL POPULATION (n=50)

Out of 50 cases of non PEM babies admitted in ICH, total number of babies with pneumonia is 12%, skin infections is 30%, other infectious fevers accounted for the rest.

Infections	Percentage
Skin infections	30
Pneumonia	12
Others	58

TABLE-11

COMMON PREDISPOSING FACTORS IN PEM (n =100)

Most of the babies with PEM had multifactorial reasons for the probable incidence of protein energy malnutrition, the commonest of which is related to breast feeding, either prolonged or early weaning. Other reasons were low socioeconomic status, twinning, bottle feeding, prematurity, poor diet and poor spacing. One other important factor for PEM is malnutrition.

TABLE- 12

COMMON INFECTIONS IN PEM (n=100).

The commonest infection seen in the study group was pneumonia 40%, followed by skin infections 30%. Urinary tract infections 22% and diarhoeas 18%.

Infections	Percentage
Pneumonia	40
Skin infections	30
UTI	22
Diarhoea	18

TABLE-13

ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE ORGANISMS

Organism	Pen	Ampi	Oxa	Erythro	Cotri	Cef	Ak	Cip	Van
Staphylococcus aureus MSSA(n =8)	100% 8	100% 8	100% 8	50% 4	100% 8	100% 8	100% 8	100% 8	100% 8
MRSA (n=2)	0	0	0	1 50%	2 100%	0	2 100%	1 50%	2 100%
Cons (n=2)	1 50%	1 50%		1 100%	1 50%	2 100%	2 100%	1 50%	1 50%

All Staphylococcus aureus were sensitive to vancomycin.

TABLE -14

ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE ORGANISMS

Organism	AMPI	COTRI	CEF	AK	CEFTAZ IDIME	GENTA	CIP	IMI
Klebsiella pneumoniae (n=24)	6 25%	8 33.3%	10 41.6%	15 62.5%	13 54.2%	14 58.35	20 83.3%	24 100%
Escherichia coli (n = 10)	6 60%	4 40%	6 60%	8 80%	6 60%	6 60%	8 80%	5 100%
Pseudomonas aeroginosa (n = 4)	1 25%		4 100%	4 100%	4 100%	1 25%	3 75%	4 100%

All organisms were sensitive to imipenum

TABLE-15

ORGANISM ASSOCIATED WITH THE MORTALITY IN INFECTIONS IN PEM

One baby with septicemia due to Staphylococcus aureus died of the underlying disease. Other babies were alive after the actual episode of infection was corrected with antibiotics.Hence the percentage of mortality in this study is 1%.

DISCUSSION

Total number of cases examined for case study of infections and infestations in protein energy malnourished babies in a tertiary care centre was 100 and other nonmolnourished children admitted in the inpatient department in ICH as a control study was 50. Similar studies has been undertaken in various hospitals in other parts of the world was also correlated.

Out of 100 babies under five examined in the hospital for PEM and various co morbid factors, 55% (55 babies) were male babies and other 45% (45 babies) were female babies. In Dhaka shishu hospital, the ratio was nearly the same as 58:42 male: female.

Out of the 100 children examined, 22% babies were under the age of six months and another 78% babies were of age from 6months to five years. Of these, nearly 50% of the babies were under the age of two years.

When the control population was also taken into consideration, sever infections were found in 74% of protein energy malnourished babies and 44% of control group. This shows that protein energy malnutrition is a definite predisposing factor for with the deficit in macronutrients and general loss of immunity in this population of babies.

Out of 100 babies with PEM examined, 43% (43 babies) had grade I PEM, 30% (30 babies) had grade II and 26% (26 babies) had grade III

PEM. This correlates with the Dhaka Shishu Hospital study which showed 44% grade I, 29% grade II PEM.

Out of the 100 babies examined for disease states, 44 babies showed signs of clinical pneumonia and their sputum sample and endotracheal aspirate was collected and examined for growth of organisms. The results showed growth of Klebsiella 2.5% cases.

Out of 100 babies examined, 18 babies showed clinical signs of diarrhea with some, moderate dehydration. Their stool sample was cultured for the possible growth of organisms and one stool sample showed no growth. The majority of the samples growed Escherichia coli (66.6%), salmonella 22.2% and Klebsiella 11.1%.

Total number of babies who showed signs of fever with painful micturition or blood in urine are 22, out of 100 and their urine sampled. Growth showed the following pattern. Out of 22 babies, Escherichia coli was grown in 72.7% cases, Klebsiella pneumonia in 18.1% and Proteus mirabilis in 9.09% cases.

Skin infections in the form of perianal absesses, pyoderma in the babies under five years of age in study accounted for total of 28 cases, out of which Staphylococcus aureus grewin 57.4%, Pseudomonas aurogenosa in 21.4% and Escherichia coli in 14.2% cases.

Out of two cases of septicemia admitted in intencive care unit of the hospital, one baby grew klebsiella pneumoniae accounting for 50% and the other baby grew Staphylococcus aureus accounting for other 50%. One baby with Staphylococcus infection succumbed to the disease, accounting for mortality in this case.

Out of 10 babies who had perianal inching and history of passing worms in stools, 3 babies showed Ascaris lumbricoides and one baby showed Strongyloides stercoralis in the wet mount and iodine mount for parasite examination.

Most common infections in the control population of 50 cases, pneumonias in 12% and skin infections in 30% babies. Other common infections in this group seemed to be acute upper respiratory infections, ottitis media etc.,

Regarding antibiotic sensitivity for gram positive organisms, Staphylococcus aureus was sensitive to pencillin, ampcillin, oxacillin, cotrimoxazole, cefotaxime, amikacin, ciprofloxacin and 50% were sensitive to erythromycin. Regarding Coagulase Negative Staphylococcus aureus, ciprofloxacin, and erythromycin was sensitive in 50% cases and cotrimoxazole was sensitive in all cases.

Regarding gram negative organisms, sentivity pattern is as follows: Klebsiella pneumonia was sensitive to ampicillin in 25% cases,

cotrimoxazole in 33.3%cases, cefotaxime in 45.6% cases, amikacin in 62.5% cases, ceftazidime in 58.35%cases.

Regarding Escherichia coli, 60% were sensitive to ampicillin, cefotaxime, ceftazidime and gentamicin. 40% sensitivity was seen in case with cotrimoxazole and 80% with amikacin. With pseudomonas, 100% sensitivity is seen with cotrimoxazole, cefotaxime and amikacin. 255 sensitivity was seen with ampicillin. All the culture growths were sensitive to imipenum.

SUMMARY

- Protein energy malnutrition arises from misbalance of protein and energy requirements of the baby and actual attainment.
- PEM leads to various infections and infections aggravate PEM.
- In PEM, there is a decrease in immunity levels and defict of macronutrients which aggravate the chances of acquiring infections.
- In this study conducted with hundred babies with PEM, 55% male babies and 45% were female babies.
- 22% of the PEM babies were under 6months of age and remaining from 6months to 5 years.
- 43% of the babies had grade I PEM, 30% grade II PEM and 26% grade III PEM according to IAP classification.
- Out of 100 babies examined, 40% babies had pneumonia, most commonly due to Klebsiella pneumonia 62.5%.
- 18% of the babies had clinical signs of diarrhea, most common organism isolated being Escherichia coli 66.6%.
- Out of 22 babies with urinary tract infections, Escherichia coli occurred to be the commonest infecting organism 72.7%.
- 28% of the babies examined had skin infections, commonest organism isolated being Staphylococcus aureus.

- 2% of the babies had septicemia, one each due to Klebsiella and Staphylococcus aureus.
- Parasitic infections were seen in only 4 babies, three showed Ascaris and one with Strongyloides.
- This is seen as against common control group, where 12% babies had pneumonia and 30% babies showed signs of skin infections along with other minor infections.
- Drug sensitivity pattern does not show any major change in both the control and the study group.
- All the gram positive organisms studied were sensitive to vancomycin and all the gram negative organisms studies were sensitive to Imipenum.

CONCLUSION

Protein energy malnutrition increases the chances of a baby to get infections more than nonPEM babies and these infections tend to follow severe course in these babies, important factor being decrease in immunity. This forms a vicious cycle, infections increasing the severity of Protein energy malnutrition. In this study group, most common infection seen was pneumonia followed by skin infections, urinary tract infections and diarhoea. This is as against the control group, where most common infection seem to be skin infections followed by minor respiratory infections. Antibiotic sensitivity pattern in both the study and control group does not change in a large way showing increase incidence of infections in the malnourished babies than normal babies.

PROFORMA

RISK FACTOR PROFORMA

- name
- Age
- Sex
- Address
- Education
- Religion
- Symptoms of PEM
- Socioeconomic status
- Referring centre

PREDISPOSING FACTORS OF PEM –PROFORMA

- Bottle feeding
- Maternal malnutrition
- LBW
- Prematurity
- Twins
- Malformations
- Artificial feeding
- Prolonged breast feeding
- Early weaning

- Food fads
- Infections
- Immunization status
- Poor diet
- Poor spacing
- Working parents
- Pregnancy in mother
- Living conditions
- Ignorance poverty

CLINICAL HISTORY PROFORMA

- H/O PEM
- Birth history if relevant
- H/o focal infections or infestations
- H/o associated risk factors
- H/o recurrent infections if any

TO EXAMINE FOR COMMON INFECTIONS

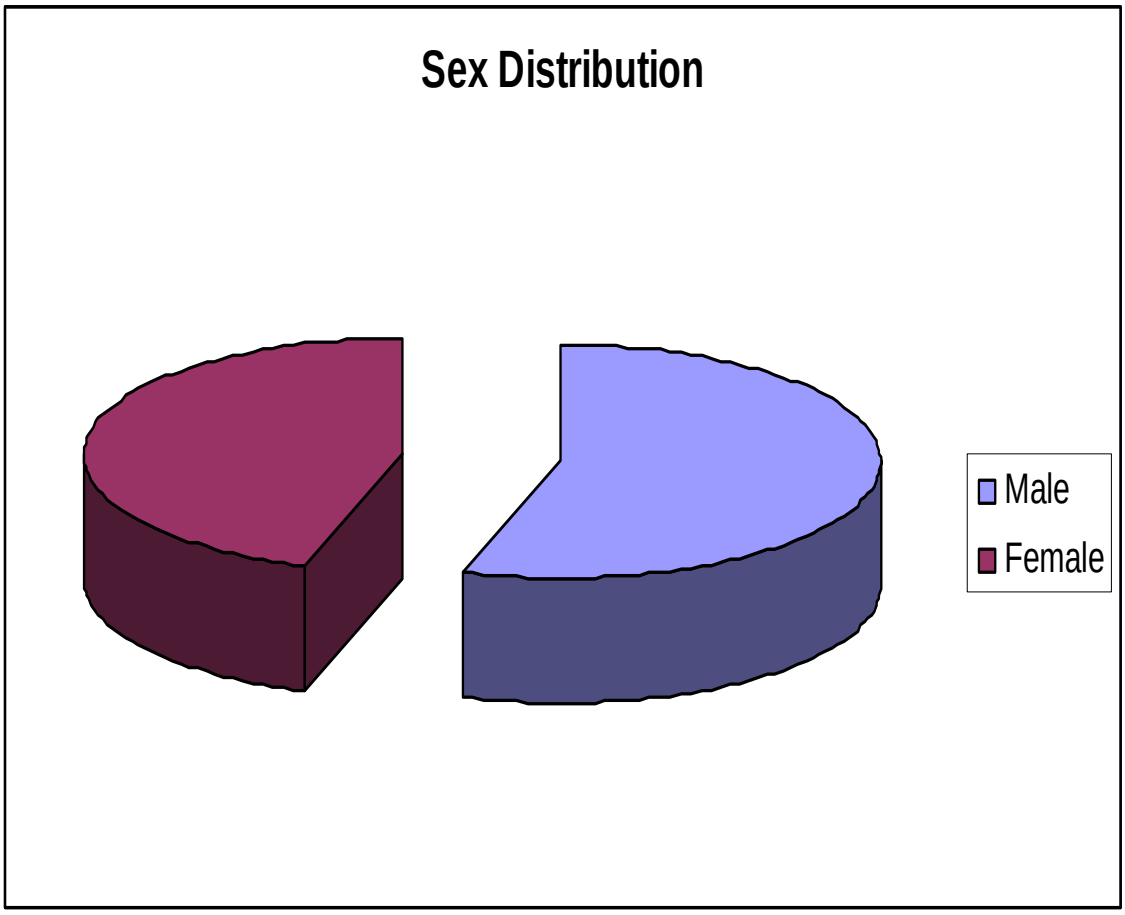
- Pneumonia
- Otitis media
- UTI
- Diarhoea

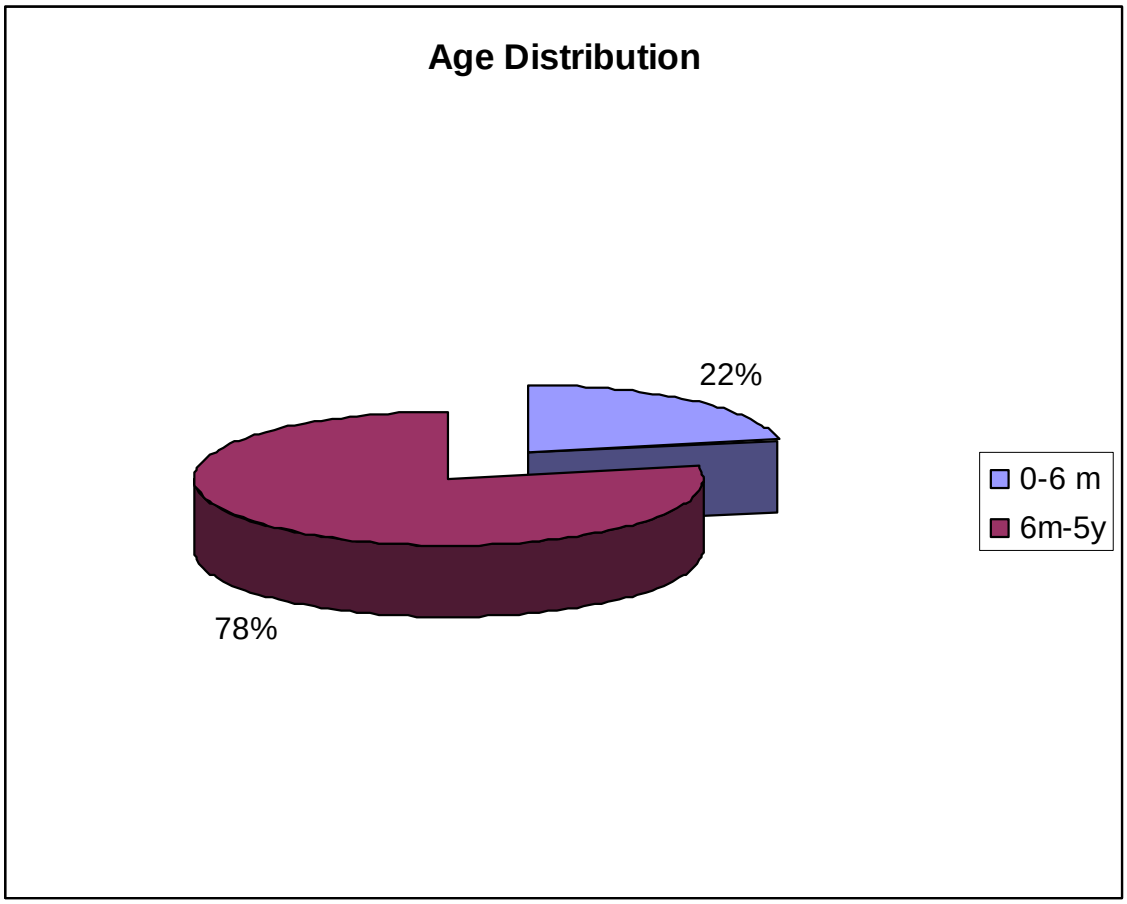
- Skin infections
- Parasitic infections

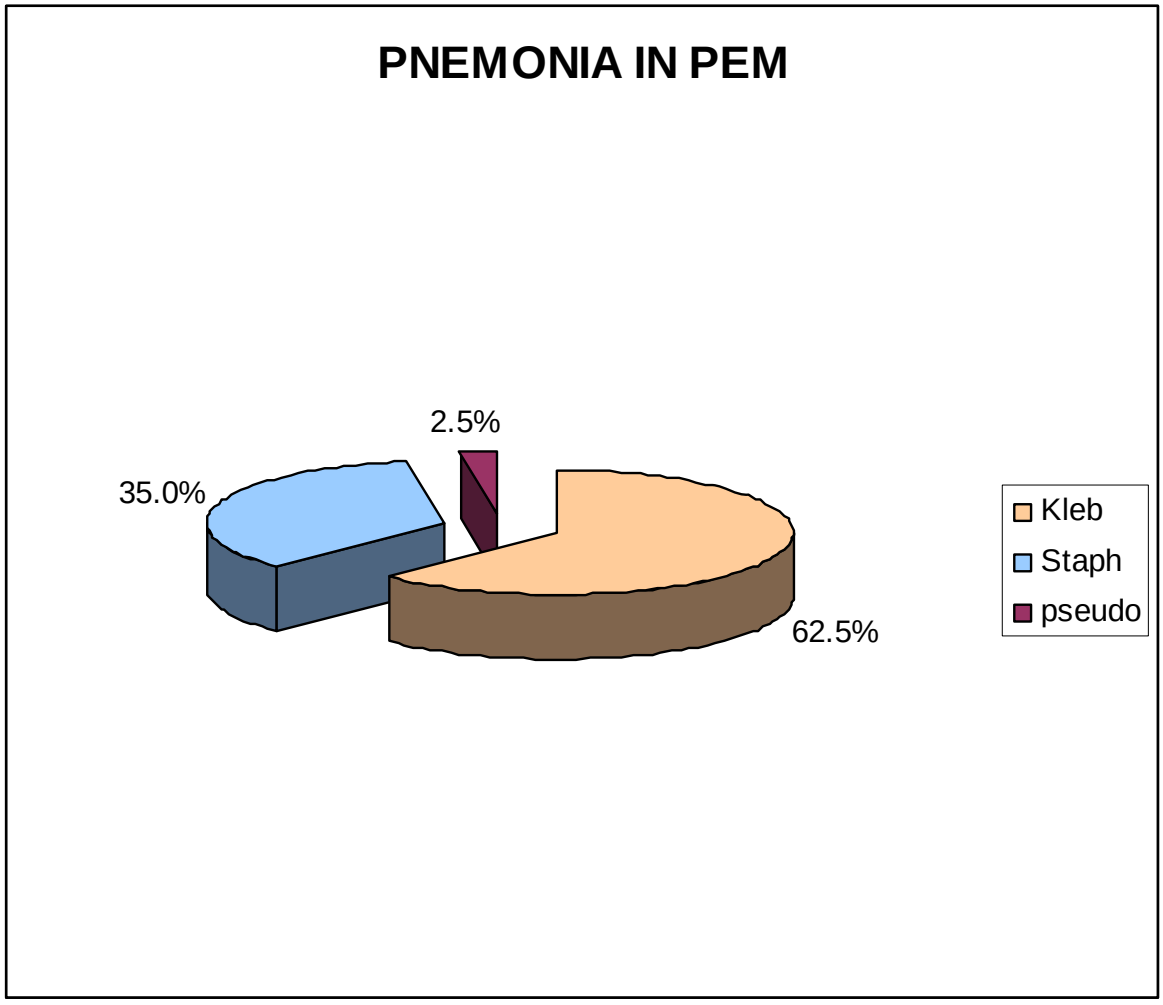
Sample Collection:

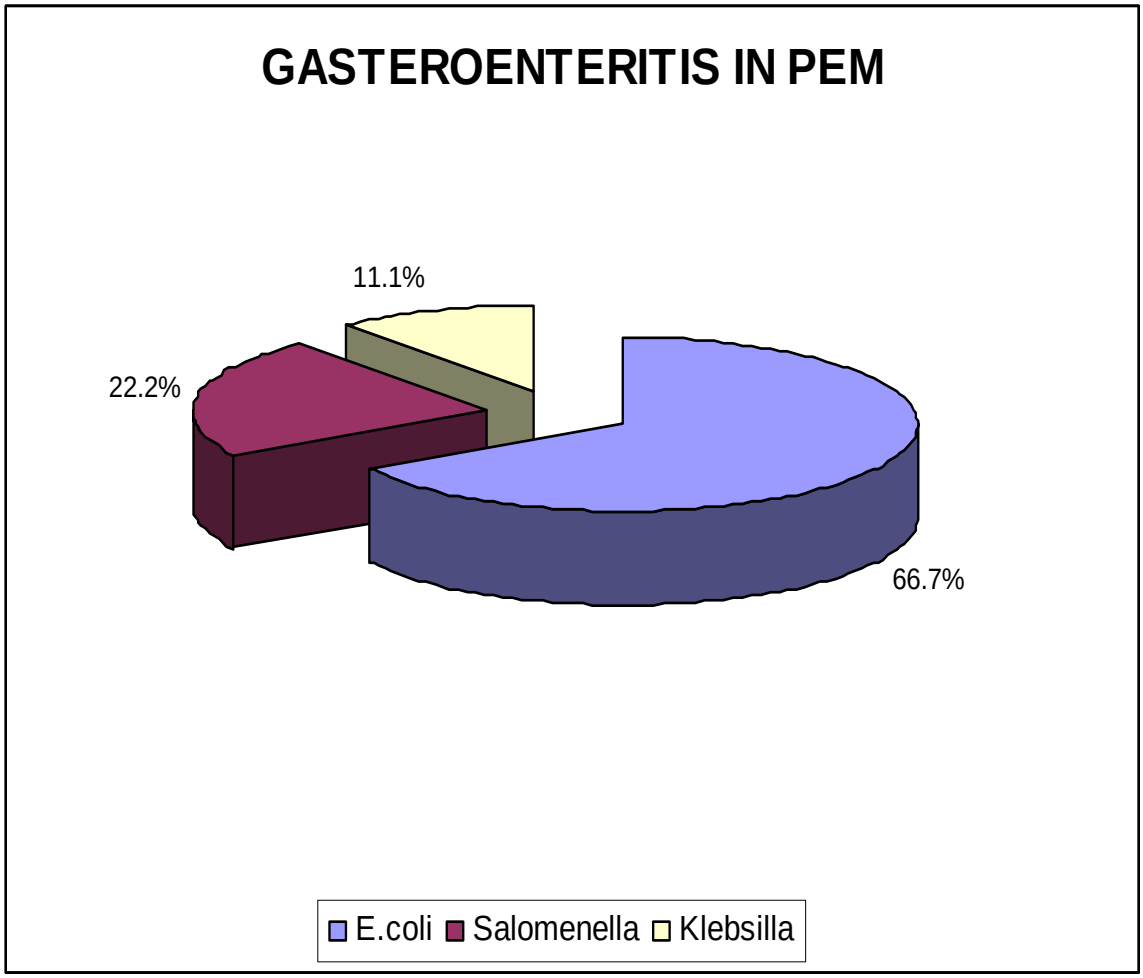
Sample processing.

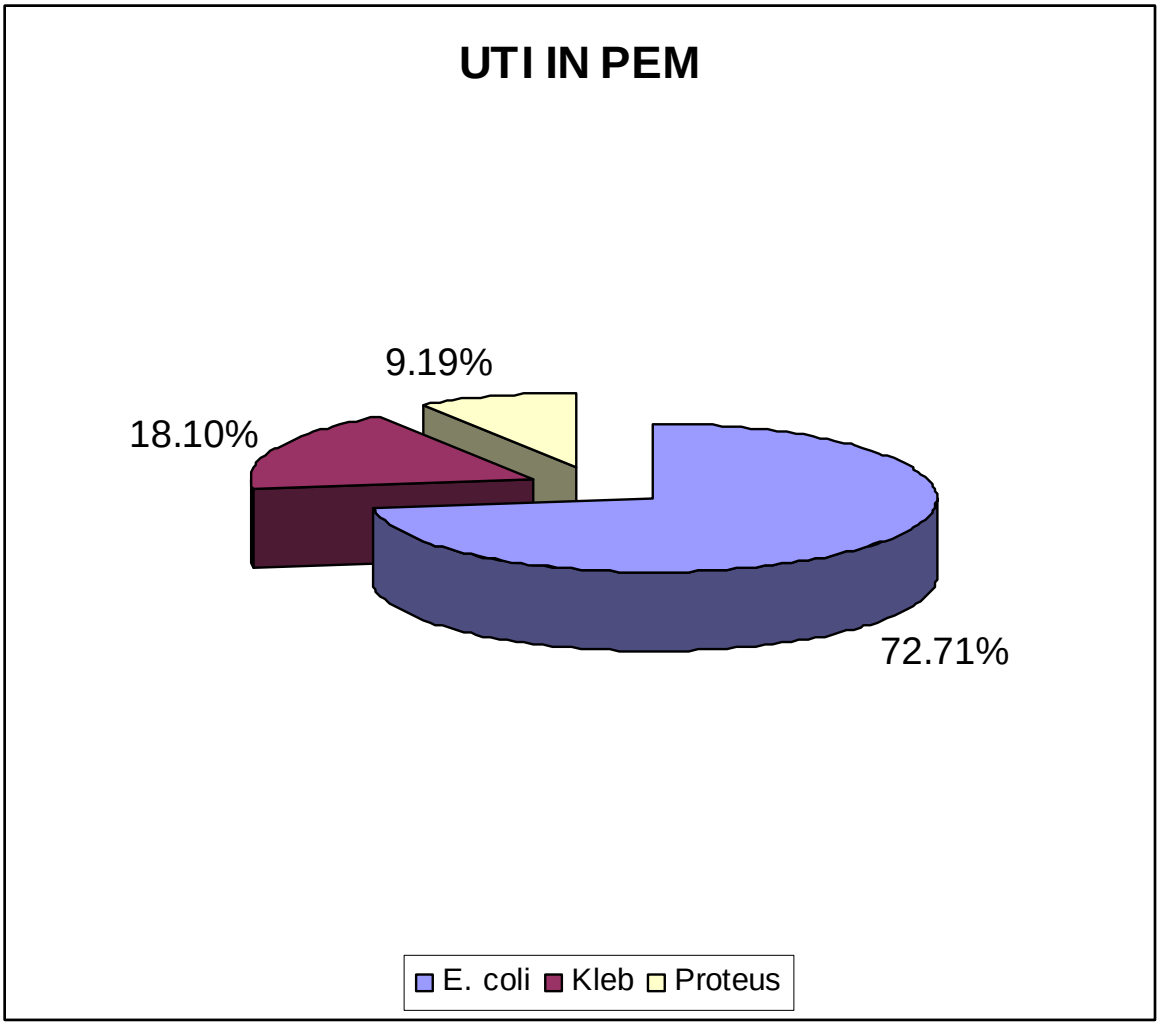
APPENDIX

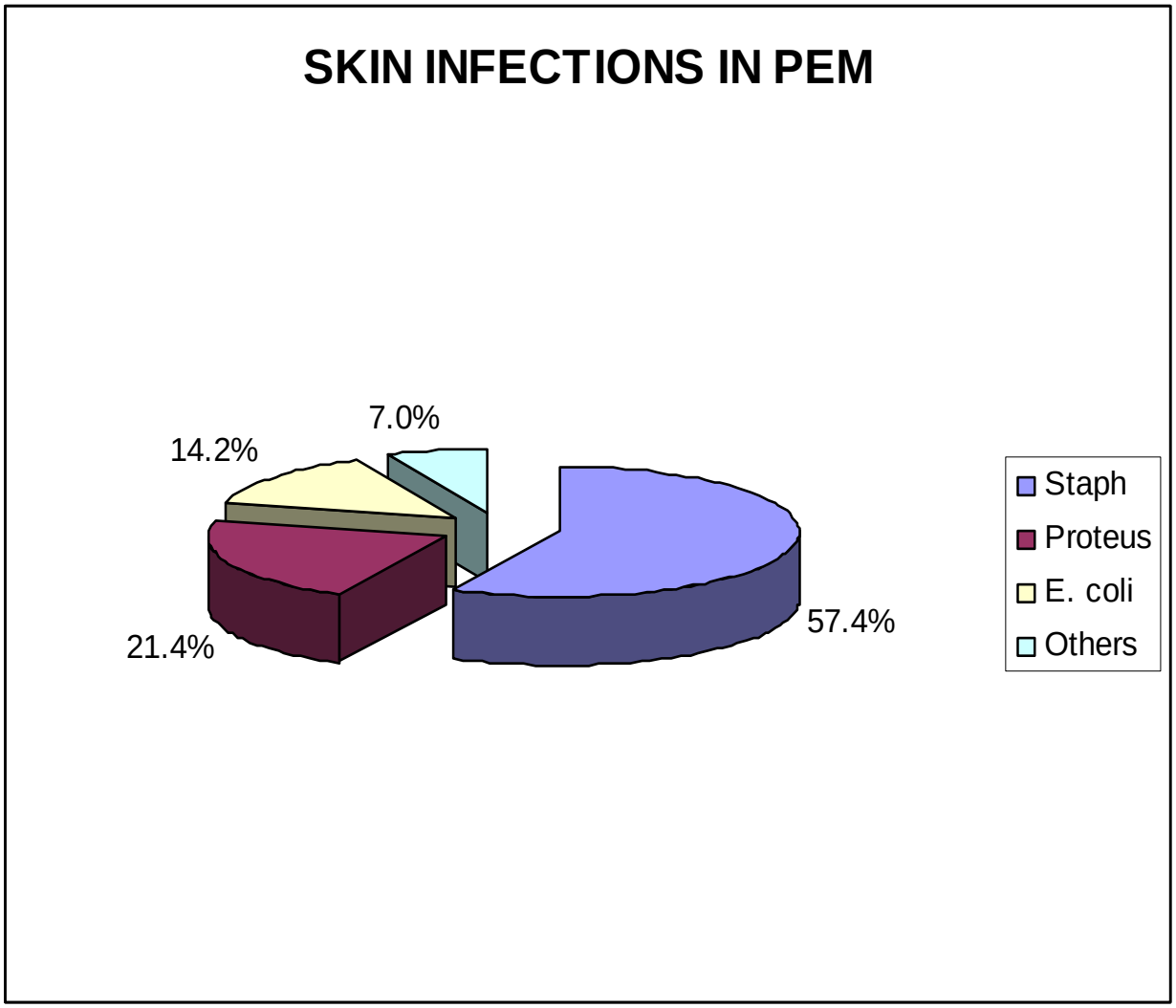

Sex Distribution
Male
Female

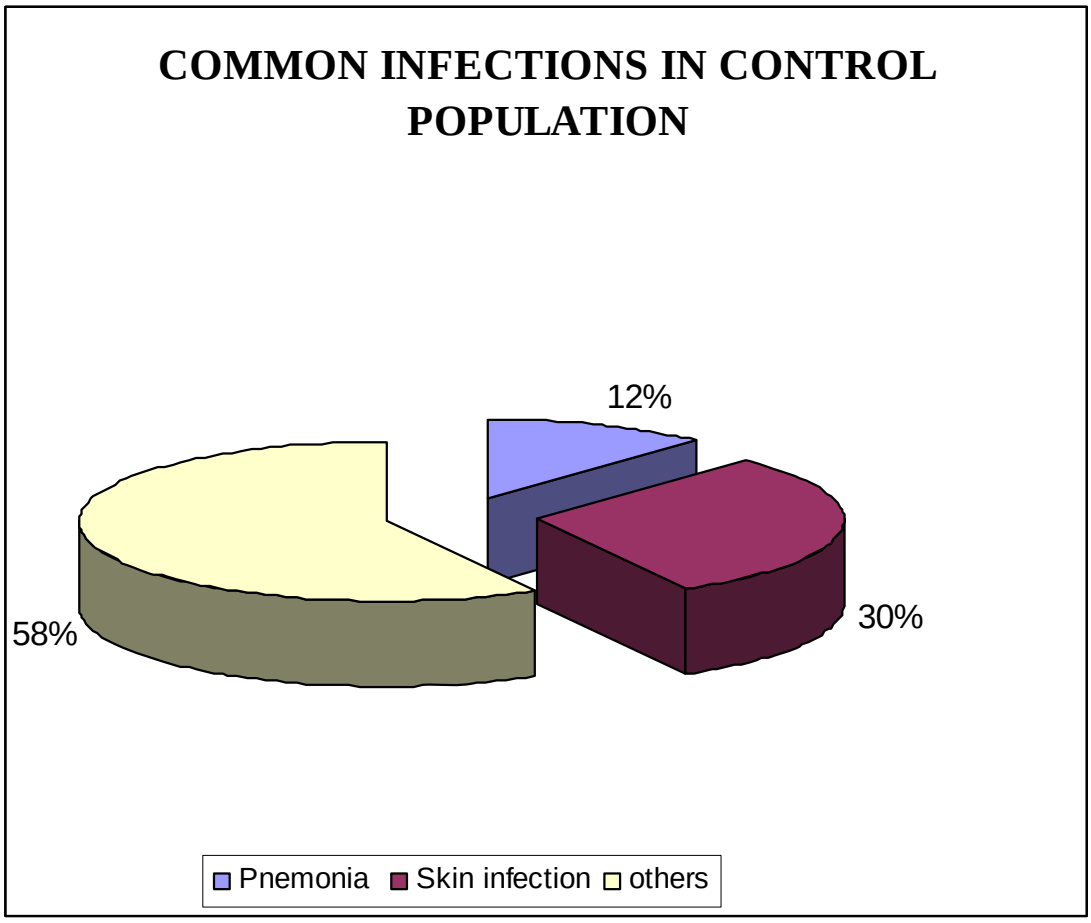

Age Distribution
22%
78%
0-6 m
6m-5y


PNEMONIA IN PEM
2.5%
35.0%
62.5%
Kleb
Staph
pseudo

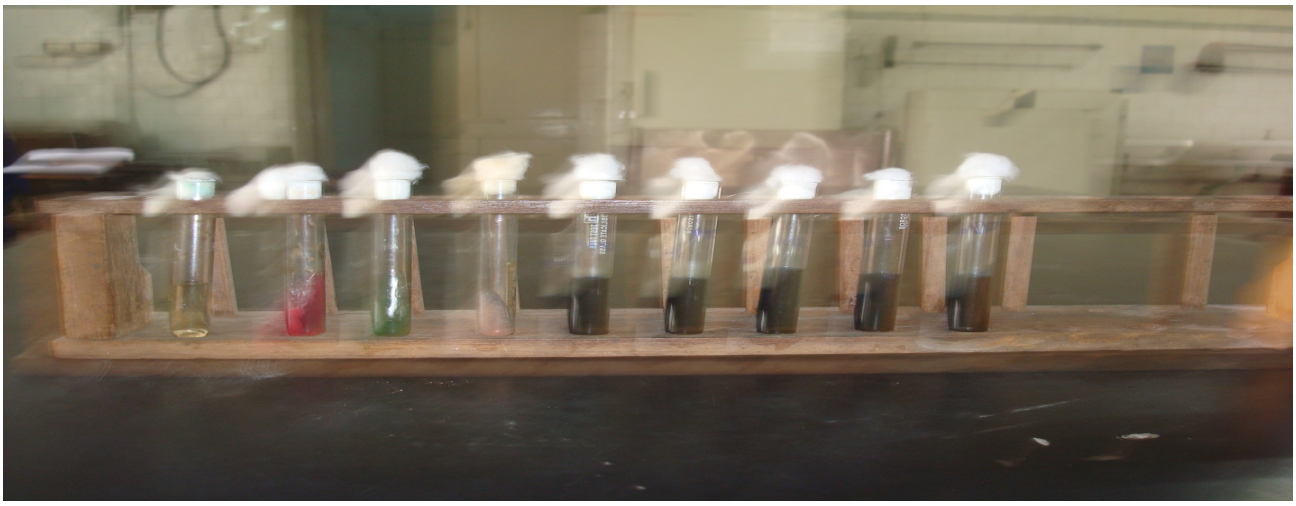

GASTEROENTERITIS IN PEM
11.1%
22.2%
66.7%
E.coli
Salomenella
Klebsilla


UTI IN PEM
9.19%
18.10%
72.71%
E. coli
Kleb
Proteus


SKIN INFECTIONS IN PEM
7.0%
14.2%
21.4%
57.4%
Staph
Proteus
E. coli
Others


COMMON INFECTIONS IN CONTROL POPULATION
12%
30%
58%
Pnemonia
Skin infection
others

COLOR PLATES

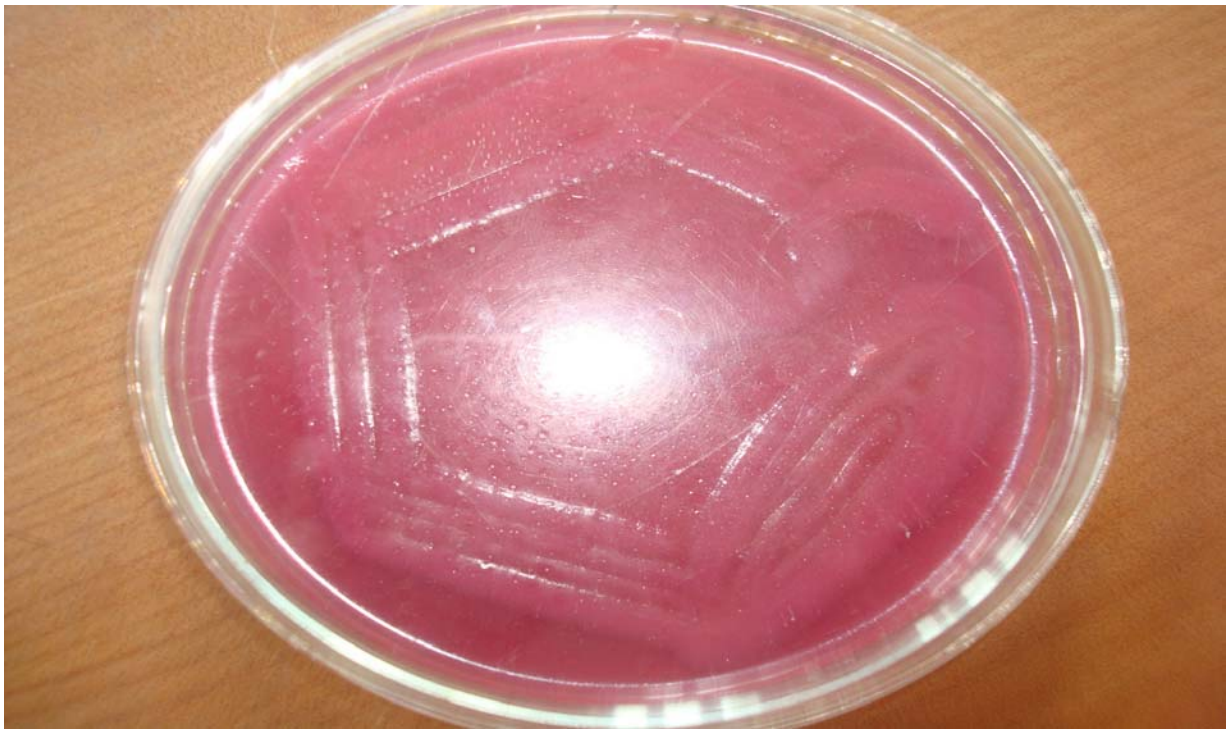


Pseudomonas auerogenosa reactions

Culture plate of Pseudomonas aureogenosa

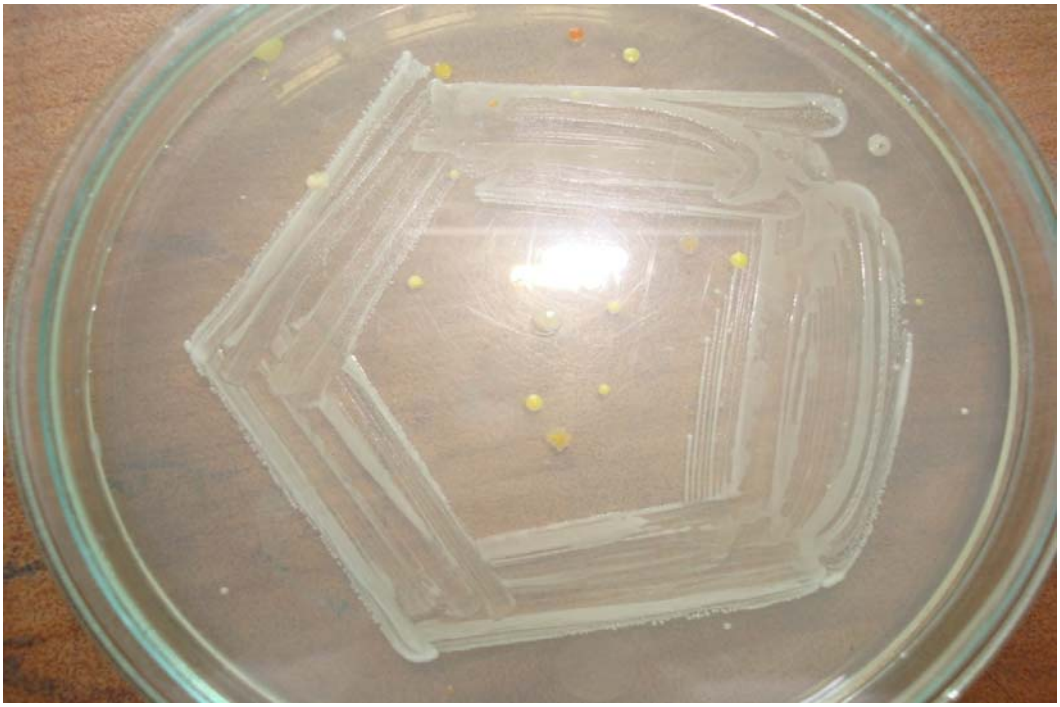
Salmonella typhi reactions

Escherichia coli reactions



Colony of E.coli

Klebsiella pneumonia culture



Culture of Staphylococcus aureus

Reactions of Klebsiella pneumonia

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MEDIA AND STAINING METHODS USED

APPENDIX - I

CULTURE MEDIA

BRAIN HEART INFUSION BROTH

Sodium citrate lgm

Sodium chloride 4gm

Sodium phosphate 5gm

Dextrose l0 gm

Peptone 1 0gm

BRAIN HEART INFUSION

Brain infusion broth 250ml

Heart infusion broth 750ml

Sodium polyanethol sulphonate 0.25gm

Obtain Ox brain and heart. Remove all fat from the heart. Cut into small pieces and grind. Add distilled water three times and keep at 4°C overnight. From the brain, remove meninges fully and then, weigh. Add distilled water and mash by using hand. Keep in the cooler over night. Next morning boil the brain and heart separately for 30 minutes. Then filtered through cotton layer. Measure each broth separately. Mix both infusions and the remaining ingredients. Dissolve well and adjust Ph of the entire amount to 7.4 to 7.6.

Autoclave at 121° C for 15 minutes. Filter through filter paper and distribute in screw-capped bottles in 50 to 100ml amounts. Autoclave once more at 115° C for 10 minutes.

ROBERTSON'S COOKED MEAT MEDIUM

Beef infusion broth

Minced and dried meat

Fat and connective tissue were removed from lean meat. Meat is mixed with 2 parts of water and refrigerated over night and any remaining fat is skimmed off. Boiled for 30 minutes. The mixture is filtered through 2 layers of gauze and the meat particles were dried. pH is adjusted to 7.4 to 7.6. The dried meat particles were distributed in 12 X 100 mm test tubes to a height of 1.5 to 2.5 cm .The pH adjusted filtrate was then added to get 3-4 parts liquid per tube. The tubes were plugged and sterilized by autoclaving.

BLOODAGAR

Sterile Defibrinated sheep blood -7 ml

Nutrient Agar (melted) -100ml

Nutrient agar was cooled to about 45-50 °C, 5-7ml of sterile defibrinated sheep blood was added. Mixed well and 15 ml of blood agar was poured in petri dishes.

CHOCOLATE AGAR

Sterile defibrinated blood - 10 ml

Nutrient Agar (melted) -100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

MACCONKEY AGAR

Peptone - 2 gm

Sodium chloride - 2 gm

Bile salt - 0.5 gm

Lactose - 1 gm

Agar - 1.5 gm

Distilled water - 100 ml

All the ingredients except lactose were dissolved in distilled water by heating. pH adjusted to 7.6.1 ml of 1% neutral red solution was added to every 100 ml of medium with lactose and sterilized by autoclaving at 121°C for 15 minutes.

MEDIA FOR BIOCHEMICAL IDENTIFICATION OF BACTERIA

Oxidase Catalase

Indole Triple Sugar Iron agar

Citrate Urease

Methyl Red – Voges Proskauer Nitrate reduction

Phenyl alanine deaminase test Carbohydrate fermentation

Media

1. OXIDASE REAGENT

Tetra methyl P - phenlene diamine dihydro chloride -1% aqueous solution.

2. CATALASE TEST

3% Hydrogen peroxide

3. INDOLE REAGENT

Kovac's reagent

Para dimethyl amino benzaldehyde - l0gm

Iso amyl alcohol - 150ml

Hydrochloric acid - 80 ml

4. TRIPLE SUGAR IRON AGAR

Sodium chloride 0.5gm

Yeast extract 0.5gm

Peptone 2gm

Agar 1.5gm

Distilled water 100ml

Distilled by keeping in boiling water bath and the following

ingredients were added.

Lactose l.0gm

Sucrose l.0gm

Dextrose 0.1 gm

Sodium thio sulphate 0.03gm

Ferrous sulphate 0.02 gm

pH adjusted to 7.6. Phenol red 0.0024 gm (2.4 ml of 1% solution) was added and distributed into test tubes in 4 ml quantities and\ autoclaved. The tubes were kept in a slanting position so that to get a deep butt and a short slant.

5. SIMMON'S CITRATE AGAR

Sodium Chloride 5.0g

Magnesium sulphate 0.2g

Ammonium dihydrogen phosphate l.0g

Potassium dihydrogen phosphate l.0g

Sodium citrate 5.0g.

Agar 20.0g

Bromothymol blue (1/500 aqueous solution) 40 ml

Distilled water 100 ml.

The ingredients were mixed and pH adjusted to 6.9.Sterilized byautoclaving and poured into tubes as slopes.

6. **CHRISTENSEN'S UREA AGAR**

Urea Solution

Sodium Chloride -5.0g

Dextrose -l.0gm

Trypticase -l.0gm

Mono potassium phosphate -2.0gm

Urea -20.0gm-

Distilled water -100ml

Phenol red 1% solution -1.2 ml (in alcohol)

Urea agar base

Agar -1.5 gm

Distilled water - 90 ml

The ingredients were dissolved in distilled water. pH adjusted to 6.8. Phenol red solution was added & sterilized by filtration. This is stock solution. Agar was dissolved in distilled water and sterilized by autoclaving. Cooled to 45° C and 10 ml of urea solution was added, dispensed in 3-5 ml quantities and allowed to form a small butt and a long slant.

7. GLUCOSE PHOSPHATE BROTH (MR-VP medium)

Dipotassium phosphate 5.0gm

Glucose 5.0gm

Distilled water 100ml

The above ingredients were suspended in distilled water and heated slightly to dissolve them. Sterilized at 11 5° C for 1 5 minutes.

8. POTASSIUM NITRATE BROTH

Potassium nitrate (KN03) 0.2gm

Peptone 5.0gm

Distilled water 100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

9. PHENYL ALANINE DEAMINASE TEST

Yeast Extract 3g Dl-Phenylalamine 2 g

Disodium hydrogen phosphate lg Sodium Chloride - 5 g

Agar 12g Distilled water - 1 lr

pH adjusted to 7.4, distributed in tubes and sterilized by autoclaving at 121° C for 1 5 minutes, allowed to solidify as long slopes.

MEDIA FOR TESTING ANTIBIOTIC SENSITIVITY PATTERN

Mueller - Hinton Agar

Beef extract 20.0gm

Acidicase peptone 7.5gm

Starch l.5gm

Agar 17.0gm

Distilled water 1000ml

The ingredients were dissolved in one liter of distilled water, mixed thoroughly. Heated with frequent agitation and boiled for 1 minute. pH adjusted to 7.4 +/0.2.Sterilized by autoclaving and poured m plates.