Epidemiology of Gastroenteritis in Saudi Arabia.

Thesis submitted for the degree of Doctor of Philosophy
at the University of Leicester.

by

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STATEMENT

This thesis is based on work conducted by the author in the Department of Microbiology and Immunology of the University of Leicester, U.K. and the King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia during the period between May 1992 and March 1995.

All the work recorded in the thesis is original unless otherwise acknowledged in the text by references. None of the work has been submitted for another degree in this or any other University.

Date 24/3/95

DEDICATION

To my Mother who despite her failing health has supported and believed in me over the years. She remains a source of inspiration. To my son Zain who has had to take second place during this long period of study.

University of Leicester

Abstract

Faculty of Medicine

Microbiology and Immunology

Doctor of Philosophy

Epidemiology of Gastroenteritis in Saudi Arabia.
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In order to determine the aetiology and epidemiology of gastrointestinal infections in Saudi Arabia; viral, bacterial and parasitic causes of diarrhoea at a major referral centre were examined. Bacterial enteropathogens were found in 7.7% of patients; *Salmonella* species (51.7%) were found to be the most frequent pathogens followed by *Campylobacter jejuni* (28%) and *Shigella* species (14.9%). *Clostridium difficile* was also found in 9.5% of patients examined but no correlation could be found with presence of faecal leukocytes or pH. Susceptibility patterns of 15,467 isolates of *Enterobacteriaceae* to 14 antibiotics over 6 years showed that resistance increased in all the *Enterobacteriaceae*. Imipenem and ciprofloxacin were the only agents to remain active. Protozoan or metazoan parasites were detected in 27.8% of patients examined, the most common being *Giardia lamblia* and *Hymenolopsis nana*. Of the patients tested for viruses in stools, 14% had rotavirus, 8.5% adenovirus, 1.5% SRSVs and 0.3% coronavirus. Adenoviruses in stools were detected and serotyped for the first time in Saudi Arabia. Data were correlated with clinical history and serology which showed that immunosuppression was a major factor for onset of gastroenteritis. Type 40/41 were most prevalent followed by types 1,2,3, and 5. Most infections were in children under five years. Astroviruses were detected by PCR and gave an incidence of 1.5%. Rotavirus electropherotypes were determined by polyacrylamide gel electrophoresis and exhibited mostly the long electropherotype characteristic of group A subtype II. Environmental surfaces on a hospital ward were examined over a six month period in which rotavirus was found in 7% of sites tested and equated with areas involving most human activity and occurrence of rotavirus infections in patients. Diagnostic methods such as biotinylated DNA probe and latex agglutination (LA) were evaluated for rapid detection of enteric infections. LA was found to be suitable as a screening method but the DNA probe showed very low sensitivity and specificity for diagnostic use.

Publications

Parts of the work presented in this thesis have formed the basis for the following publications:

Akhter J., Markely Burdette J., Hussain Qadri S.M.H., and Myint S.H. (1994). Aetiology of gastroenteritis at a major referral Centre in Saudi Arabia. Journal of International Medical Research; 22:47-54.

Akhter J., Hussain Qadri S.M.H., and Myint S.H. (1994). Comparison of a biotinylated DNA probe with current methods for the detection of adenovirus in diarrhoeal disease. Medical Science Research; 22:477-478.

Akhter J., Min S., Hussain Qadri S.M.H., and Myint S.H. (1995). Diagnosis of astrovirus by reverse transcriptase PCR in stools from patients in the U.K. and Saudi Arabia. Clinical Diagnostic Virology; 3:215-220.

Akhter J., Hussain Qadri S.M.H., Myint S.H. (1995). Adenovirus Infections. Infectious Disease Practice for the Clinician, Jan:1-4.

Akhter J., Hajjar S., Myint S.H., and Hussain Qadri S.M.H.. Viral contamination of environmental surfaces on a general paediatric ward and playroom in a major referral center in Riyadh. European Journal of Epidemiology (In Press).

Akhter J., Hussain Qadri S.M.H., Lee G.C., and Myint S.H.. Changing patterns of antibacterial resistance in clinical isolates of *Enterobacteriaceae* at a tertiary care center in Saudi Arabia. Saudi Pharmaceutical Journal; 3(3)

Akhter J., Hussain Qadri .M.H., and Myint S.H.. Epidemiology of gastrointestinal adenovirus infections in Saudi Arabia. European Journal of Microbiology and Infectious Diseases. (In press).

Akhter J., Sikotra S., Qadri SMH., and Myint. (1994). Diagnosis of paediatric viral gastroenteritis at two medical centres in Saudi Arabia and the United Kingdom. Journal of Diarrhoeal Disease Research; 12:257-260.

Papers submitted for publication:

1. Akhter J., Qadri S.M.H., and Myint S.H.. Can intestinal pathogens cause false positives with the latex agglutination test for *C.difficile* toxin?

2. Akhter J., Hussain Qadri S.M.H., and Myint S.H.. Electropherotyping of Rotavirus infections at a major referral center in Saudi Arabia.

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Abbreviations.

A549	human lung carcinoma cell line
AIDS	acquired immunodeficiency syndrome
BAP	sheep blood agar plate
BBD	barbital buffered diluent
BMT	bone marrow transplant
BSA	bovine serum albumen
cDNA	complementary deoxyribonucleic acid
CF	complement-fixation
CMV	cytomegalovirus
CPE	cytopathic effect
CTA	*Clostridium difficile* toxin assay
DNA	deoxyribonucleic acid
ddNTP	dideoxynucleotide triphosphate(s)
dsRNA	double-stranded ribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetracetic acid
EIA	enzyme-immunoassay
EM	electron microscopy
FBS	foetal bovine serum
GVHD	graft versus host disease
HBSS	Hanks balanced salt solution
HEK-293	human embryonic kidney cell line
HFS	human foreskin cell line
HIV	human immune deficiency virus
ICHD	immunocompromised host disease
LA	latex agglutination
LLC-MK2	secondary rhesus monkey kidney cell line
MW	molecular weight
NCCLS	National Committee for Clinical Laboratory Standards
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PEG	polyethylene glycol
PMC	pseudomembraneous colitis
RIA	radioimmunoassay
RMK	primary rhesus monkey kidney cell line
RNA	ribonucleic acid

RSV	respiratory syncytial virus
RT-PCR	reverse-transcriptase PCR
SAF	sodium-acetate-acetic acid formalin
SCID	severe compromised immunodeficiency
SDS	Sodium dodecyl sulphate
SRV	small round viruses
SRSV	small round structured viruses
Taq	*Thermus aquaticus*
TE	Tris-EDTA buffer
TEMED	N,N,N',N'- tetramethyl ethylethylenediamine
TPB	tryptose phosphate broth
TRIS	tris(hydroxymethyl)- methylamine
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

Antibiotics

AM	ampicillin
AN	amikacin
AUG	augmentin
CAZ	ceftazidime
CF	cephazolin
CFOX	cefoxitin
CIP	ciprofloxacin
FUR	nitrofurantoin
GM	gentamicin
IMP	imipenem
NET	netilmycin
PIP	piperacillin
TE	tetracycline
TMP-SX	trimethoprim-sulphamethoxazole

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I am also grateful to my Department Head Dr. S.M. Hussain Qadri for providing me laboratory facilities and without whose support I could never have begun this thesis. I am indebted for his assistance with materials, publications and moral support.

I am poured out like water, and all my bones are out of joint: my heart is like wax; it is melted in the midst of my bowels.

Psalms 22:14

CHAPTER 1. Introduction.

1.1. Background.

Diarrhoea is derived from the Greek word 'diarrhoia' meaning to flow through. It is a common symptom of gastrointestinal disease, characterized by increased frequency and water content of stools. Infections of the gastrointestinal tract afflict people of all ages and the use of such terms as Montezuma's revenge, Delhi belly, Aztec 2-step, Greek gallop, Rome runs, Hong Kong dog, Turkey trots, Basra belly, Poona poohs and backdoor sprint illustrate its widespread occurrence. Diarrhoea is a disruption in bowel habits whereby normally formed stools passed once every day or every few days is replaced by frequent and numerous liquefied movements. Working definitions in common use and used in this thesis are (Gracey 1993):

Diarrhoea - is the passage of one watery or explosive stool, or three loose stools in 24 hours.

Dysentery - refers to loose stools admixed with blood and/or mucus.

Acute diarrhoea - refers to episodes of infectious diarrhoea with rapid onset lasting 4-5 days.

Chronic diarrhoea - lasts for 14 days or more.

Acute diarrhoea represents a non-specific response of the intestine to a number of different kinds of insults, including infections, adverse drug reactions, inflammatory bowel disease, and ischaemia. However, the major cause of acute diarrhoea in adults is infection primarily by viruses but also by bacteria, parasites and yeast (Blacklow and Greenberg, 1991; Guerrant and Bobak, 1991).

Acute infectious diarrhoeal disease is an immense worldwide problem. Billions of cases occur each year, with an estimated 10-15 million deaths directly attributable to acute diarrhoeal disease (Mitchell and Skelton, 1985). Diarrhoea is a leading cause of illness and death among children in developing countries, where an estimated 1.3 thousand million episodes and 3.2 million deaths occur each year in those under 5 years of age. Children may suffer from 3-9 episodes of diarrhoea per year (Guerrant et al., 1986). About 80% of deaths due to diarrhoea occur in the first two years of life. In less developed countries, diarrhoeal morbidity is especially severe. In Bangladesh, Black et al. (Black et al., 1982) found children under 3 years age had diarrhoea intermittently for an average of 55 days per year. This prevalence contributed to long term malnutrition and delayed growth. The disease tends to become milder as the age of the child increases due to acquired antibodies and increasing maturation of intestinal cells.

The importance of diarrhoeal disease among the elderly has been increasingly recognized in recent years. In a report from the Centers for Disease Control (CDC) that analyzed 28,538 diarrhoeal deaths in the U.S.A. between 1979-1987,

the majority of deaths (51%) occurred in adults over 74 years (Lew et al., 1991). A subsequent CDC study of 87,181 hospitalizations for which gastroenteritis was one of the top three discharge diagnoses, found that 85% of 514 diarrhoeal deaths occurred in adults 60 years or older. Age was the most important risk factor for death subsequent to a hospitalization involving gastroenteritis. The case-fatality ratio was also higher than in children (Gangarosa et al., 1992).

1.1.1. History

Diarrhoeal diseases have been important to all societies since the beginning of recorded history. Hippocrates used the term "dysentery" to denote a condition wherein the affected person, experiencing straining and painful defaecation, passed many stools containing blood and mucus. Through the First World War, outbreaks of diarrhoea and dysentery were as important in deciding the outcome of battle as were war-related injuries.

The modern era of diarrhoeal diseases began with the identification of the causative agents involved. During the mid 1800s, *Giardia lamblia* and *Entamoeba histolytica* were first identified. Then during the latter part of the nineteenth century, *Shigella* and *Salmonella* organisms were characterized, and the two forms of dysentery-bacillary and amoebic- were distinguished.

During the 1960s cholera toxin was purified and during the 1970s, enterotoxigenic *Escherichia coli* were identified as important causes of diarrhoea. This was soon followed by the discovery of other enterotoxin- and cytotoxin-producing

bacteria: *Salmonella*, *Aeromonas*, *Yersinia*, *Clostridium perfringens*, *Clostridium difficile*, enterohaemorrhagic *Escherichia coli*, and *Staphylococcus aureus*.

During the early 1970s, viruses were also clearly implicated as causes of diarrhoea in humans. Initially, Norwalk virus was shown to produce gastroenteritis in volunteers fed bacteria-free stool filtrates (Kapikian, 1972). Soon thereafter, rotaviruses were established as a major cause of infantile gastroenteritis.

1.1.2. Aetiology

Cumulative data from epidemiological studies show that approximately 20-50% of cases of diarrhoea are attributable to known bacterial or parasitic pathogens, which suggest that viruses might be responsible for the remainder. In fact, viral gastroenteritis has been found to be the second most common clinical entity in developed countries, following closely behind viral upper respiratory tract illness (Kapikian et al., 1980).

It is difficult to establish an accurate correlation between clinical features and microbial aetiology because the spectrum of clinical manifestations is wide and variable. However, certain classic features can be indicative of a particular aetiology; examples include dysentery (amoebiasis, shigellosis, enteroinvasive *Escherichia coli*), bloody diarrhoea (salmonellosis, campylobacterosis, shigellosis, EIEC), 'rice-water stools' (cholera), significant diarrhoea in a young infant (rotavirus), haemorrhagic colitis, chronic diarrhoea and flatulence (giardiasis), appendicular

syndromes (yersiniosis), and short term incubation with vomiting(staphylococcal). Many aetiological studies still do not include control data, and pathogens identified in the stools during an acute attack of diarrhoea are assumed to be the causal agent. This may not be the case, for example, the presence of *Campylobacter* in developing countries has been found to be as common in controls as in patients with diarrhoea (Rowland et al., 1986).

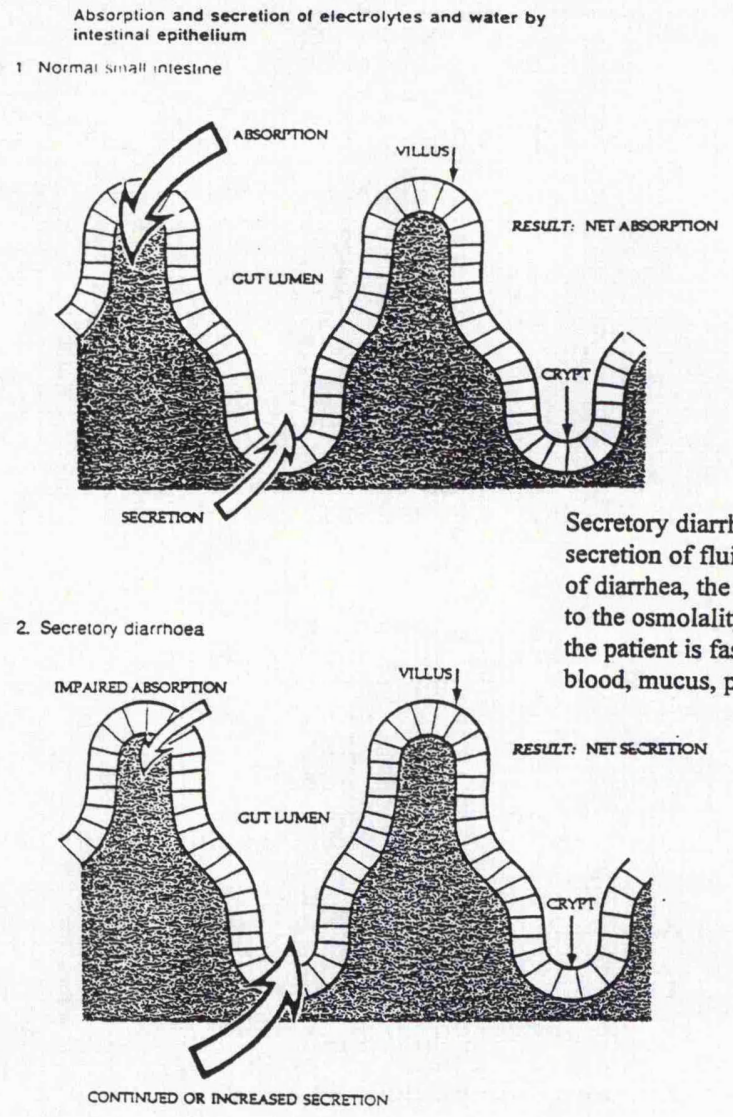
With the emergence of the acquired immune deficiency syndrome as a massive health problem throughout the world, it has become apparent that these patients are prone to gastrointestinal infections with otherwise unusual micro-organisms including *Isospora* sp, *Mycobacterium avium-intracellulare*, *Cryptosporidium parvum*, cytomegalovirus, herpes simplex, adenovirus, chlamydia and syphilis (Smith et al., 1988). In Haiti and Africa up to 95% of AIDS patients initially present with diarrhoea. In the U.S.A. 50-60% present with diarrhoea (Colebunders et al., 1987; Soave and Johnson, 1988).

1.1.3. Mechanisms of Diarrhoea.

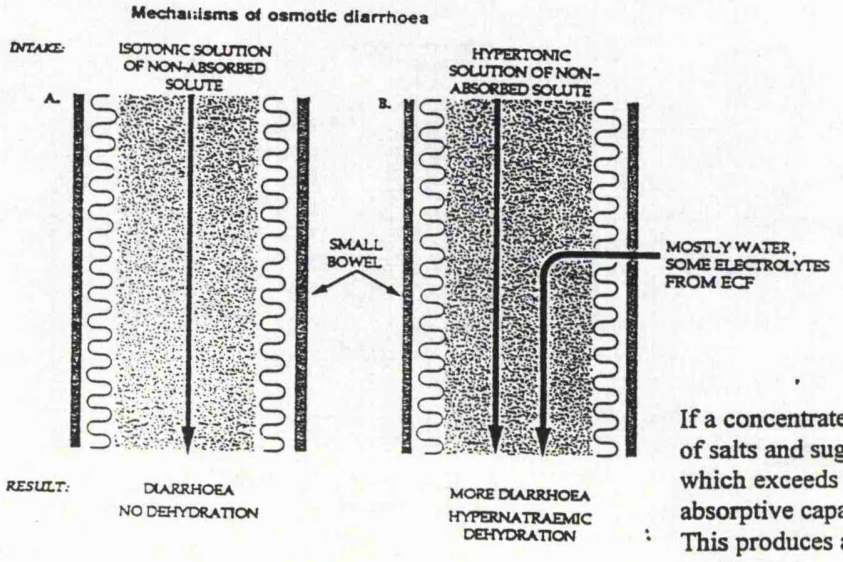
There are two principal mechanisms by which watery diarrhoea occurs: secretion and osmotic action (Fig. 1.1.3.1.). Intestinal infections can cause diarrhoea by both mechanisms, secretory diarrhoea being more common, although both may occur in an individual. Secretory diarrhoea is caused by the abnormal secretion of water into the small bowel. This occurs when the absorption of sodium by the villi is impaired while the secretion of chloride in the crypt cells continues or is

increased. This net loss of water and salts causes dehydration. These changes can also result from the action of bacterial toxins. Osmotic diarrhoea occurs when a poorly absorbed , osmotically active substance is ingested , which simply passes through causing diarrhoea.

Fig.1.1.3.1. Mechanisms of Diarrhoea



Secretory diarrhoea is mediated by the intestinal secretion of fluid and/or electrolytes. In these forms of diarrhea, the ionic content of faecal fluid is similar to the osmolality of the plasma; diarrhoea continues if the patient is fasted, and the stools do not usually contain blood, mucus, pus cells or other exudates.



If a concentrated osmotically active solution of salts and sugars or amino acids is taken which exceeds the threshold of intestinal absorptive capacity, osmotic diarrhoea occurs. This produces a loss of water more than solutes, leading to hypernatraemic states. A feature of osmotic diarrhoea is that it decreases or stops when the offending cause is removed (eg. laxative intake or nonabsorbable substrates such as mannitol or sorbitol). Malabsorption of dietary carbohydrate is a very important cause of osmotic diarrhoea in children.

Adapted from: Readings on Diarrhoea. WHO, Geneva. 1992.

Detailed mechanisms of action vary with the nature of the pathogen:

1. Viruses.

Columnar epithelial cells in the distal parts of the villi of the small intestine are the usual sites of viral replication. There is shortening of the villi, elongation of crypts, and increased number of mononuclear inflammatory cells in the lamina propria. Vomiting is due to stimulation of vagal and sympathetic afferent nerves in the medulla oblongata. Diarrhoea is characterized by profuse watery stools containing increased concentrations of electrolytes. Diarrhoea may occur by two mechanisms. The most important is the functional immaturity of villus epithelial cells; the other is due to reduction in the total absorptive area due to flattening and clumping of villi (Davidson, 1991).

Various reports have demonstrated that infection of cultured eukaryotic cells by a number of different animal viruses alter the intracellular sodium, potassium and calcium ion concentrations (Carrasco and Lacal, 1983). Healthy villous enterocytes maintain a low intracellular Na+ concentration by actively transporting the ion out of the cell using the Na+/K+ pump located on the basilateral walls of the columnar epithelia. The active transport of Na+ from the cells provides a concentration gradient for the absorption of Na+ and consequently water from the gut lumen. Glucose also moves with the Na+ into the cells where it is released and diffuses into the intercellular spaces and distributed around the body.

An increase in the levels of Na+ and a decrease in the levels of K+ ions demonstrated during viral infections has been attributed to either an alteration in the permeability of the epithelial plasma membrane or to an inhibition of Na+/K+ ATPase activity. It has also been proposed that an increase of Na+ concentration within the cells could be responsible for the observed inhibition of host cell protein synthesis during viral replication (Carrasco and Lacal, 1983). Defective Na+ transport and/or carbohydrate malabsorption, due to extensive atrophy of the villi, are considered the primary mechanisms in the establishment of diarrhoea by rotavirus infection. In viral infection leukocytes are not usually seen in the stool. Recovery occurs when the villi regenerate and the villous epithelium matures.

2. Bacteria

Bacteria that multiply within the small intestine adhere through pili or fimbriae that bind to receptors on the intestinal surface; this occurs with *Escherichia coli* and *V. cholera* 01. Some bacteria such as enterotoxigenic *Escherichia coli* produce toxins that alter epithelial cell function. These toxins reduce the absorption of sodium by the villi and increase the secretion of chloride in the crypts, causing secretion of water and electrolytes (Hart et al., 1993). Recovery occurs when affected cells are replaced by healthy ones after 2-4 days. *Shigella, C. jejuni, Salmonella* and enteroinvasive *Escherichia coli* can cause bloody diarrhoea by invading and destroying mucosal epithelial cells. This occurs mostly in the colon and the distal part of the ileum. Invasion may be followed by formation of microabcesses and superficial ulcers; hence the presence of red and white blood cells, or visible blood in the stool.

3. Parasites

Protozoa such as *G. lamblia* and *Cryptosporidium parvum* adhere to the small bowel epithelium and cause shortening of the villi. With *G. lamblia* mucosal damage and malabsorption also play a role. *Entamoeba histolytica* causes diarrhoea by invading epithelial cells in the colon or ileum and causing microabcesses and ulcers. This only happens when the infecting strain is virulent. In about 90% of human infections the strains are non virulent; in such cases there is no mucosal invasion and no symptoms occur, although amoebic cysts and trophozoites may be present in the faeces.

1.1.4. Epidemiology.

Patterns of infectious micro-organisms associated with diarrhoea differ geographically and according to whether patients are hospitalized or not. In colder or temperate climates admissions for acute diarrhoea are mainly attributed to rotavirus. In Scandinavia, for example, most episodes occur in Winter or Spring. In Bangladesh, bacterial pathogens account for 24% of diarrhoeal stools. In studies in Somalia and Egypt ETEC, rotavirus, *Salmonella*, *Shigella*, *Aeromonas*, *Campylobacter*, *Cryptosporidium parvum* and *V. cholera* non-01 were the main isolates found. At least 16 million people travel annually from industrialized countries to developing countries. Around one third of these suffer travellers diarrhoea, 30% having to be confined to bed.

1.1.5. Transmission

The mode of transmission of aetiological agents of diarrhoea helps determine the epidemiological patterns which occur. The main route is faecal excretion followed by oral ingestion involving fingers, flies, fluids, foods and fomites. Factors such as overcrowding, hygiene practises, inadequate sanitation, sewage disposal and environmental contamination are major determinants of infection.

Nosocomial transmission is among the most common of reported diarrhoea outbreaks reported to the Centers for Disease Control and accounts for 21% of all 223 nosocomial outbreaks reported from 1956-1979 (Stamm et al., 1981). In recent years day-care centres have become increasingly recognized as reservoirs of infectious agents such as respiratory tract viruses, hepatitis A, *Staphylococcus, Streptococcus*, *Haemophilus* and enteric pathogens. The incidence of diarrhoea in children under 3 years of age in day care-centres is estimated to be 3.5 illnesses per child per year.

1.1.6. Aim of Study

Saudi Arabia is a fast developing, newly industrialized country. It has undergone a rapid transformation from a nomadic lifestyle to a modern state in only a 50 year span. Saudi Arabia is also unique in that almost one third of the population are expatriate workers from all parts of the world. Hence, much basic knowledge needs to be obtained on the nature of many infectious agents. This is particularly true of viral infections due to the dearth of virology laboratories in the country.

The aim of this study is to review the main causes of gastroenteritis due to viral, bacterial and parasitic causes at a prominent referral centre as a basis for establishing relevant aetiologies and their epidemiology. However, the main emphasis will be on viral infections and adenoviruses in particular where no prevalence data is available. I will also look at some diagnostic methods and their applicability to epidemiological studies.

1.2. Enteric Viruses

The modern era of the laboratory study of diarrhoea of viral aetiology began in 1972 with the use of immune electron microscopy by Kapikian et al. (Kapikian et al., 1972), which demonstrated the causative virus of a large outbreak in Norwalk, Ohio. During the 1970s various viruses were discovered in the stools of patients with diarrhoea. These include rotaviruses, adenoviruses, Norwalk, caliciviruses, astroviruses, reoviruses and possibly coronaviruses. The common enteroviruses are associated with relatively few cases of viral gastroenteritis.

Electron Micrographs of Enteric Viruses.

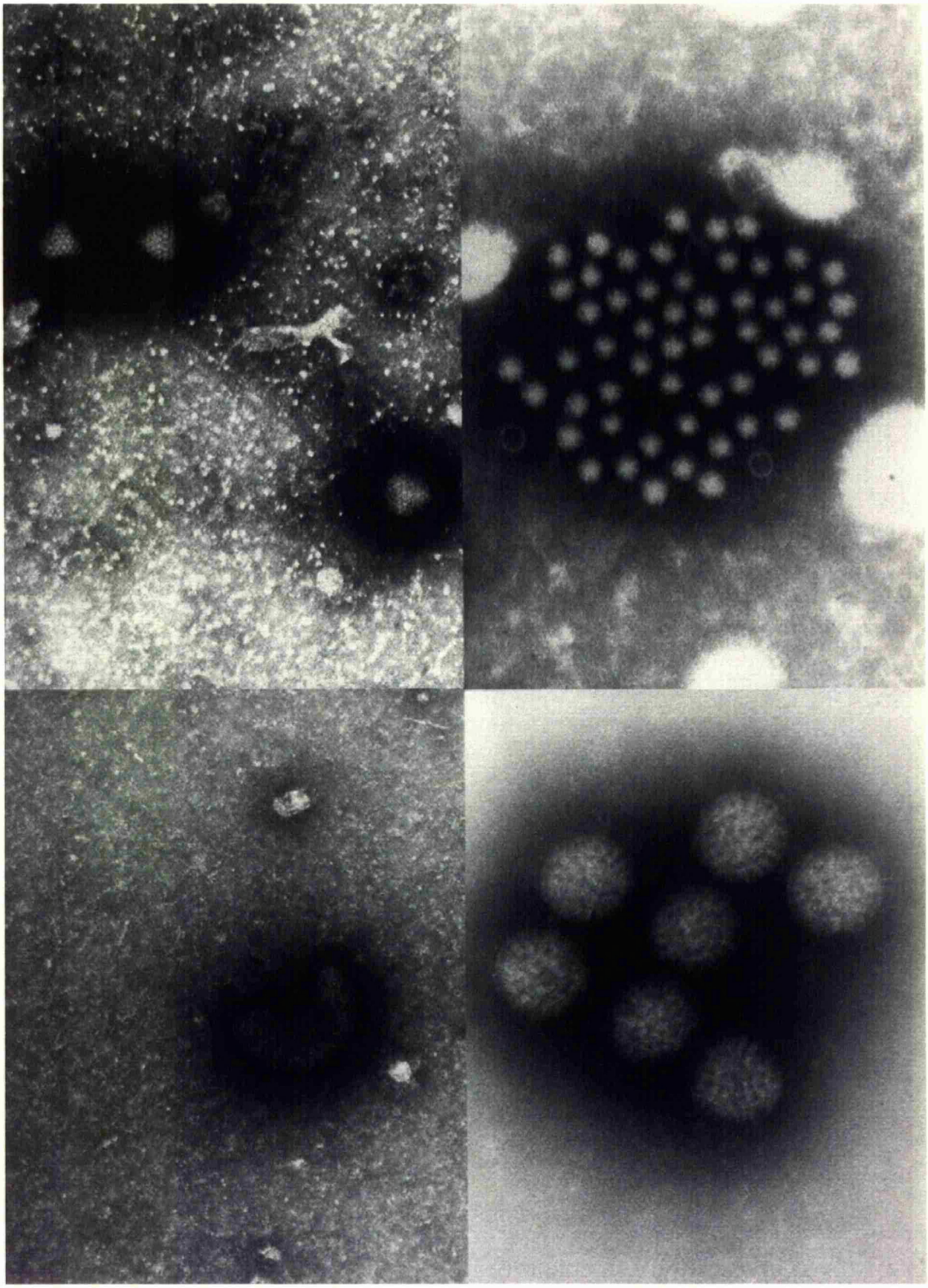
All samples were negatively stained for 1 minute in 0.5% phosphotungstic acid (pH6.0) and visualized using a Jeol electron microscope. Photographs taken at King Faisal Specialist Hospital and Research Centre with the kind help of Mr. Jeffrey McClintock.

The electron micrographs of the following viruses are shown:

1. Adenovirus - magnification x 140,000
2. Astrovirus - magnification x 140,000
3. Coronavirus - magnification x 100,000
4. Rotavirus - magnification x 140, 000
5. Enterovirus (tissue section) - magnification x 100,000
6. Cytomegalovirus (tissue section) - magnification x 100,000

Adenovirus

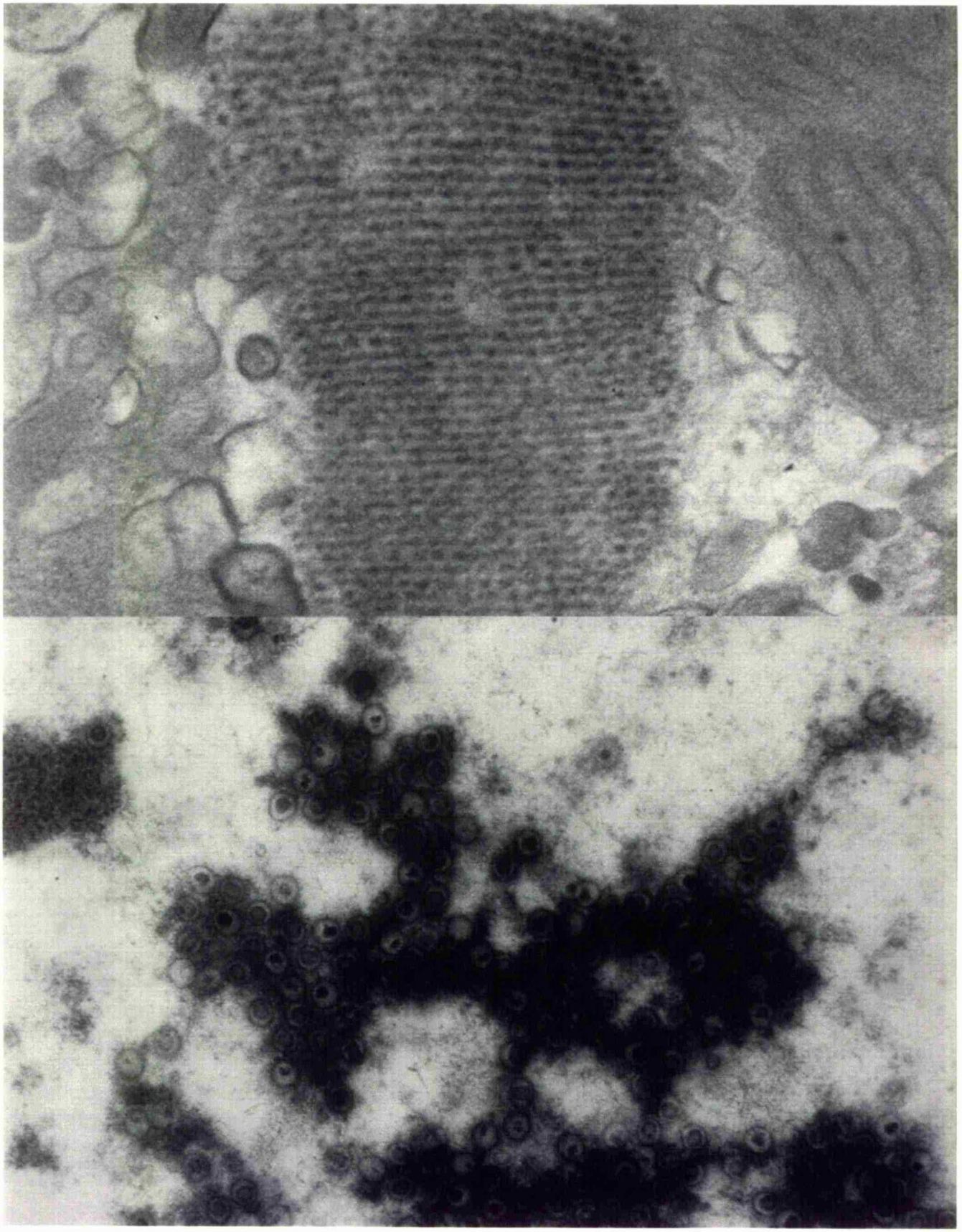
Astrovirus



Coronavirus

Rotavirus

Enterovirus



Cytomegalovirus

1.2.1. Rotavirus.

In 1973 Bishop et al. (Bishop et al., 1973) examined intestinal biopsy specimens to discover rotaviruses. They are now known to be the leading worldwide cause of serious viral enteritis in infants and young children. Rotavirus strains have been found to infect avian and most mammalian species tested, including piglets, infant mice, calves, goats, lambs, rabbits, foals, dogs, turkeys, chickens and monkeys (Wyatt et al., 1982). Early attempts to classify the rotavirus genome by ELISA, immunoelectron microscopy, and complement-fixation demonstrated a common antigen which has subsequently been mapped to VP6, the major inner capsid polypeptide. Subsequent use of plaque reduction neutralization assays identified VP7 as the major outer capsid antigen. Because the subgroup and neutralization antigens are genetically and morphologically distinct, a classification scheme was devised based on subgroup specificity associated with VP6 and serotype specificity associated with VP7. The outer membrane protein, VP4 is encoded by RNA segment 4. This protein has several functions, including haemagglutination activity, a role in virulence in mice, and induction of protective antibody-mediated immunity. VP4 is cleaved into VP5 and VP8 by proteolytic enzymes, which leads to enhanced activity by permitting direct penetration of the virus through the cellular plasma membrane (Matsui et al., 1989).

More recent investigations have documented the existence of antigenically distinct rotaviruses that do not share common group antigens. Thus, on the basis of distinct antigens, rotaviruses have been divided into six groups 'A to F'. The initially recognized rotaviruses have been designated group A. The non-group A rotaviruses (B-F) have been

isolated from a number of mammalian species (Jashes et al., 1986).

Rotaviral illness is a significant cause of morbidity and mortality in the developing world, accounting for 6% of all diarrhoeal episodes and 20% of all diarrhoea-associated deaths of children under 5 years of age. In the U.S.A. rotavirus infects virtually every child by the age of 4 and causes over one million cases of severe diarrhoea and 35-40% of hospitalized diarrhoea (Brandt et al 1983). This high morbidity but low mortality can be attributed to effective oral rehydration therapy. Rotavirus infection in the non-immune host generally results in profuse diarrhoea, usually with vomiting, fever and/or respiratory illness. In severe cases, rapid dehydration can result in renal failure and death. Outbreaks are common in infants and young children in hospitals and the virus can rapidly spread within families. The elderly, and patients hospitalized for the treatment of other conditions, also have a high risk of acquiring rotavirus.

Outbreaks are rare in adults due to the universal acquisition of serum antibody to these viruses at an early age. The high levels of antibody are maintained throughout adult life suggesting that subclinical infections may occur to maintain immunity. In general, the only adults who are susceptible to symptomatic infections have either been exposed to a high inoculum, are debilitated or otherwise immunocompromised. Weiss and Clark (1985) have shown that rotaviruses are rapidly inactivated at pH 2.0, the acidity of the normal fasting stomach. Infant pH levels tend to be about 3.2 compared to a pH of 1.0 in adults, which may also explain why rotavirus disease occurs largely below the age of 3 years.

A study at the Children's Hospital National Medical Center, Washington D.C. demonstrated the occurrence of subclinical infections in adults (Kim et al., 1977). 55% of adult household contacts of children hospitalized with rotavirus gastroenteritis developed serological evidence of infection, at the same time of child admissions. Only 3 of the adults (12%) developed a symptomatic illness.

Morphology.

The morphology of intact particles is distinctive and suggestive of a wheel with a core forming a hub. The average diameter is 70nm, consisting of two shells (capsids) surrounding a central core. Capsomeres radiate from the inner to the outer capsid like spokes in a wheel. The complete particle appears to be the infectious particle, whereas the inner shell is associated with the group specific antigen (Elias,1977). The proteins of rotavirus include three inner capsid proteins (VP1, VP2, VP6), three outer capsid proteins (VP3, VP7, VP9), and four non-structural proteins.

Rotavirus Genome.

The rotavirus genome of 11 segments of double strand (ds) RNA is contained within the virus core capsid. In general, the 11 ds RNA genome segments of the group A rotaviruses comprise of four high M.W. segments (1-4), five middle-sized segments (5-9), including a distinctive triplet segment (7-9) and two smaller segments (10 and 11). Analysis of the electrophoretic mobility of these segments

produces a pattern, or electropherotype. The electropherotype is usually constant for individual virus strains hence, the technique has become popular for molecular epidemiology studies to monitor outbreaks and transmission. However, viruses of different serotypes at times have the same electropherotype. But using this method type 2 rotaviruses can be distinguished from other human serotypes by their slow migrating RNA segments 10 and 11.

Laboratory Diagnosis.

Detection of virus by EM is still the mainstay for diagnosis. Group A rotaviruses are usually present in very high numbers in faeces 10^6 - 10^{10} particles per gram of stool. It is impossible to differentiate betwen the atypical rotaviruses and the group A rotaviruses by EM as they are morphologically indistinguishable. Samples positive by EM and negative for group A by serological methods suggest an atypical rotavirus infection. Immunoelectron microscopy (IEM) can be used for the direct visualization of an antigen-antibody complex. IEM improves the sensitivity of virus detection or to serotype viruses. It can also be applied to unpurified virus preparations. Electron dense colloidal gold particles can be used to further enhance the visibility of antigen-antibody interactions. Colloidal gold particles can be easily reproduced by chemical reduction of tetrachloroauric acid. Biochemical identifiers such as immunoglobulins and protein A can be electrostatically adsorbed to gold particles and these complexes used to detect and identify antigens and antigen-antibody reactions.

EIA and LA exploit the presence of group A antigen which is then detected using antibodies. However, such techniques are not suitable for the diagnosis of atypical rotavirus infections which lack the group A antigen.

Rotavirus diagnosis by detection of viral dsRNA by SDS-PAGE (electropherotyping) has also been found to be a useful method for the epidemiology of rotavirus infections, since the patterns of the disease and rate of transmission can be monitored.

1.2.2. Adenovirus.

Morphology

Adenoviruses are non-enveloped, regular icosahedrons (20 triangular surfaces and 12 vertices) that are 70-90 nm in diameter. The protein coat (capsid) is composed of 252 subunits (capsomeres), of which 240 are hexons and 12 are pentons. Each of the pentons contains a penton base on the surface of the capsid and a fibre projecting from the base. The length of the fibre varies with the adenovirus serotype. The core contains the linear dsDNA with a M.W. of 20×10^6 to 25×10^6. The hexon contains group-specific determinants and type specific epitopes. The vertex capsomere is a toxic factor that detaches cells in culture; and the fibre possesses haemagglutinins. Adenoviruses contain 13% DNA and 87% protein, have no membranes or lipids, and are therefore stable in solvents such as ether and ethanol.

Introduction

The human adenoviruses were discovered from tissue cultures of human adenoid tissue. Rowe et al. (1953) recognized that a transmissable agent was causing degeneration of the epithelial-like cells; similar viral agents were isolated from febrile military personnel with a variety of respiratory illnesses. These viruses were first called adenoid degeneration, adenoid-pharyngeal conjunctival and acute respiratory disease agents. The current nomenclature of adenoviruses was subsequently adopted in 1956.

They comprise at least 47 serologically distinct serotypes divided into six subgenera (A-F) and have been recovered from virtually every human organ system (Fig.1.2.2.1). Adenoviruses were amongst the first viral agents associated with non-bacterial gastroenteritis. Adenovirus infection is more common in infants with diarrhoea. In the 1960s (Moffett et al.,1963) these viruses were detected in 19% of infants under 2 years age with diarrhoea compared to 5% of controls. In recent years type 40 and 41 of subgenus F have been shown to cause enteric infections, which are second only to rotaviruses as a cause of infantile diarrhoea. The onset of diarrhoea is sudden and usually lasts a mean of 9-12 days (Uhnoo et al., 1984).

Epidemiology.

Schmitz et al. (Schmitz et al., 1983) summarized the world data reported to the World Health Organization on adenoviruses and associated disease between 1967 and 1976. In children up to 14 years a total of 16,548 infections were reported during the 10 years; nearly 60% involved the respiratory tract. Most of the remaining 40% were associated with CNS, gastrointestinal and unspecified febrile illnesses. A considerable proportion of these viruses were isolated from the gut, although the illness occurred elsewhere. Surveillance studies such as those in New York (Fox et al., 1968) and Seattle (Fox et al., 1977) have shown that asymptomatic spread of adenoviruses in the community is common.

The importance of adenoviruses in acute gastroenteritis in developing countries

has not been well documented. Studies in some developing countries have shown prevalence rates of 2% in Brazil (Leite et al., 1985) and Thailand (Herrmann et al., 1988), 6.5% in South Africa (Kidd et al., 1985), 12% in Mexico (Puerto et al.., 1989), and 13.2% in Africa (Tiemessen et al., 1989).

Respiratory symptoms have also been associated with gastroenteritis in which adenoviruses have been detected in patients stools. In one study, 21% of patients with adenovirus type 40/41 also had respiratory symptoms, that included tonsillitis, pharyngitis, otitis, coryza or cough. Patients who had diarrhoea due to other adenovirus types i.e. types 1-39, had diarrhoea of shorter duration and high fevers, and 79% had respiratory symptoms. Thus enteric types not only cause gastrointestinal disease, but can also be associated with respiratory disease. Sero-epidemiological studies have indicated that infection with enteric adenoviruses occurs worldwide. Studies on serum samples for enteric antibodies have shown the incidence to be 20% in children 1-6 months and 50% in children 37-48 months. Forty-eight percent of adults and 10% of the aged also have antibodies (Shinozaki et al., 1987).

Infections in the immunocompromised host constitute a new and severe problem magnified by the AIDS epidemic and escalation in organ and tissue transplantations. Adenoviruses take advantage of an impaired immune system to set up infections that are persistent, difficult to treat and can rapidly overwhelm the patient resulting in death. Adenovirus infections in immunocompromised patients are associated with case fatality rates as high as 60% in those with pneumonia and

50% in those with hepatitis (Hierholzer et al., 1992).

In patients with severe combined immunodeficiency syndrome (SCID) adenoviruses cause generalized infections that can be severe. Type 31 has been found in SCID patients with pneumonia, hepatitis, diarrhoea and acute haemorrhagic cystitis. Types 1, 2 and 5 cause respiratory, hepatic, and gastrointestinal illness and type 41 has been found in infants with gastroenteritis (Johnson et al., 1991).

In bone marrow transplant recipients lung, liver and gastrointestinal disease are the most common. Types 1, 2 and 5 predominate. Also, types 11, 34 and 35 have been associated with cystitis and nephritis in adults following bone marrow transplantation (Ambinder et al., 1986).

Case fatality rates vary from 44-60% in bone marrow transplant patients to18.4% in liver transplant patients. Adenovirus has been found to cause active infection in 12% of patients with clinical AIDS of which 45% resulted in death. Immunosuppression may also contribute to the diversity of serotypes. This may explain the unusual frequency and variety of intermediate strains in the gastrointestinal tract of AIDS patients (Maddox et al.,1992).

Laboratory Diagnosis.

Direct EM is a useful means for detecting enteric viruses such as adenovirus and is the standard procedure by which other methods are compared. In the acute phase of adenovirus enteritis, the finding of one or more virions per minute of viewing provides a strong presumptive indication of adenovirus 40/41. Specimens found to be negative by direct EM can be shown at times to be virus positive after ultracentrifugation to concentrate the virus.

EIA techniques are the most sensitive methods routinely used in laboratories by virtue of the availability of commercially prepared kits and equipment. Many EIA results can be obtained in 2-4 hours but are usually only capable of detecting group specific antigens. This applies to LA and immunofluorescence tests.

Adenoviruses 40/41 were considered non-cultivatable but Takiff et al.(Takiff et al., 1981) demonstrated that they readily grow in Graham 293 cells. Other serotypes also grow readily in A549 cell line, so that most serotypes can be isolated. in culture. They can then be identified by neutralization tests using monoclonal serotyping reagents (rabbit antisera).

Typing.

Less frequently used methods include gel electrophoresis of adenovirus DNA after restriction endonuclease treatment, which can be used for distinguishing type 40 and 41. Dot blot hybridization can detect less than 20 pg of adenovirus DNA in stools but will not distinguish serotype 40 from 41.

A recent innovation has been the polymerase chain reaction (PCR) which is an *in vitro* method for primer-directed enzymatic amplification of specific target DNA sequences. A few copies can be amplified to a level detectable by gel electrophoresis or Southern blot hybridization. The sensitivity and speed of operation may allow for routine detection of adenovirus infections (Allard et al., 1992).

Table. 1.2.2.1. Adenovirus Infections and Serotypes Involved.

Adenovirus serotypes			*Signs and Symptoms*	
Frequent	**Less Common**	**Illness**	**Frequent**	**Less Common**
31,40,41	1,2,5,12-7,21,25,26,29	Gastroenteritis	Diarrhoea, Fever	Nausea, Vomiting, Mild URI
11,34,35	1,2,5,7,21,29,30,31,37-39,4,3,45	Immuno compromised	URI, Diarrhoea pneumonia	Hepatitis, cystitis, otitis media
7	3,32	Neurologic disease	Meningitis	Encephalitis, Reye's syndrome
11	7,21,34,35	Cystitis	Cystitis (hemorrhagic)	Fever, pharyngitis
1,2,3,5,7	4,6,11,18,21,29,21	Upper respiratory illness (URI)	Coryza, pharyngitis, fever, tonsilitis	Rash, gastroenteritis
3,4,7,21	1,2,5,35	Lower respiratory illness	Bronchitis, fever, cough, pneumonia	Bronchitis
4,7	3,14,21,35	Acute respiratory disease	Fever, myalgia, coryza, tracheobronchitis	Pneumonia
5	1,2,3	Pertusis syndrome	Paroxyntial cough, vomiting cyanosis	Fever, URI
8,19,37	3,4,7,10,11,21	Keratoconjunctivitis	Keratitis, headache	Coryza, pharyngitis, gastroenteritis
2,19,.37	1,5,7,11,18,31	Veneral disease	Ulcerative genital lesions	cervicitis, urethritis

Adapted from: Adenoviruses, J.C. Hierholzer (Chap 88) In: Lennette, HE et al. Viral, Rickettsial and chlamydial infections. 6th ed (American Public Health Association, Washington, DC)

1.2.3. Astrovirus

Appleton and Higgins (Appleton and Higgins,1975) were the first to describe astroviruses by EM. Astroviruses are small (26-28 nm diameter), consisting of a five- or six-pointed surface star. They contain a positive-sense single stranded RNA genome, with a size of 7.9 kb. At least five antigenically distinct strains of astroviruses have been identified with evidence for two more new serotypes reported recently in Oxford (Lee and Kurtz, 1994). Astroviruses have been associated with outbreaks of diarrhoea in pre-school children, hospital wards and adults. They can also occur asymptomatically in neonates.

The incubation period for astroviruses is 3-4 days, followed by symptoms of vomiting, diarrhoea, headache, malaise and abdominal pain. Duration of symptoms is generally 2-3 days but can persist for a week. Dual infections with rotavirus are not uncommon, and in children with SCID, excretion of several enteric viruses including astrovirus may occur. From strains collected over a 10-year period, serotype 1 appears to be the most prevalent in the U.K., accounting for 77% of the astroviruses. The remaining types 2-5 had a similar prevalence ranging from 5-7% (Greenberg and Matsui, 1992).

1.2.4. Calicivirus

Caliciviruses are recognized by typical cup-shaped depressions on their surface and size (30-40nm). Human caliciviruses were first described in stools of children with diarrhoea in 1976 (Madeley and Cosgrove, 1976) and have since been documented in outbreaks of gastroenteritis in infants and children, antibody to calicivirus is acquired between 6 months and 2 years, and is present in 90% of older children and adults (Greenberg and Matsui, 1992).

Purification of virus protein indicates that Norwalk virus and Hepatitis E virus are similar to calicivirus. Multicentre collaborative studies suggest that there is also serological cross-relatedness between some calicivirus strains and the Norwalk virus (Cubitt et al., 1987)

1.2.5. Cytomegalovirus.

Gastrointestinal CMV disease is an erosive or ulcerative process that can occur at any location in the gastrointestinal tract. CMV infection of columnar epithelial cells, endothelial cells, myocytes and fibroblasts causes tissue destruction and ulceration. Serious CMV infection most frequently occurs with immune deficiency, after organ transplantation, in patients with cancer and after steroid therapy (Wilcox et al., 1990).

Cytomegalovirus (CMV) has been identified in the gastrointestinal tract of nearly 8% of patients with HIV, and in 13% of patients with AIDS (Frances et al 1989).

Its most common enteric manifestation is colitis, characterized by diarrhoea, abdominal pain and fever (Smith et al., 1992). Infection may occur in many areas of the gastrointestinal tract, including stomach, small intestine, appendix, pancreas and biliary tree.

Hackman et al. (Hackman et al., 1994) studied 54 consecutive upper gastrointestinal endoscopies in marrow graft recipients to determine the incidence of CMV infection in symptomatic patients. At each endoscopy 3 biopsies were obtained from the oesophagus, stomach and duodenum. Enteric CMV was identified in 52 of 486 biopsies (11%) from 14 infected patients. All patients had nausea and vomiting and 13 had oesophageal CMV associated with stomach and duodenal involvement.

1.2.6. Coronavirus.

The association of coronaviruses as causative agents in human gastroenteritis has not yet been clearly established. Although some studies have correlated the presence of coronavirus-like particles (CVLP) in diarrhoeal stools (Payne et al., 1986) other studies have found control groups to have a higher prevalence than diarrhoeal specimens (Caul and Egglestone, 1982). These viruses are 80-150 nm in diameter and are round or pleomorphic with club-shaped projections known as peplomers. Most of these viruses are associated with respiratory infection.

1.2.7. Enteroviruses.

Echoviruses and coxsackieviruses are potential viral causes of epidemic infantile diarrhoea. Echovirus 18 was isolated from 10 of 12 infants who had watery non-inflammatory diarrhoea in a 21-patient premature nursery. The virus was also isolated from two nurses, one of whom was implicated in the spread of the agent to five other babies in the ward (Eichenwald et al., 1958). There are conflicting interpretations of the significance of isolation of enteroviruses among controls as well as patients but some have suspected that they cause summer gastroenteritis.

1.2.8. Reoviruses

Human reoviruses were first isolated in the early 1950s and have been found to be ubiquitous in Nature. Reoviruses are found primarily in the gastrontestinal tract, but have also been isolated from respiratory tract secretions. They have been detected in healthy humans and in patients with a variety of gastrointestinal and respiratory illnesses. However, there has been no clearly defined role for reoviruses in these illnesses.

1.3. Enteric Bacteria

Diarrhoeal disease due to bacteria can be divided into two distinct forms; invasive and non-invasive. Invasive bacteria include *Salmonella, Campylobacter, Shigella* species. These bacteria penetrate and damage the intestinal mucosa and are associated with fever, cramps and rarely bacteraemia. They account for

approximately 15% of acute diarrhoea cases (Carpenter, 1980). Non-invasive bacteria comprise of *Vibrio cholerae, Escherichia coli, C. perfringens,* and *S. aureus.* These pathogens form enterotoxins and cause the small bowel to produce fluids in quantities that overwhelm the absorptive capacity of the colon, resulting in isotonic fluid loss.

1.3.1.*Salmonella* - These are the most ubiquitous of the bacterial pathogens, with poultry providing the greatest reservoir of infection in the U.S.A. and Europe. Aproximately 2,000 serotypes occur throughout the world and are now grouped under the umbrella of a single species, namely, *Salmonella enteritidis.* Salmonellosis is characterized by fever, cramping abdominal pain and diarrhoea (Turnbull, 1979).

1.3.2.*Campylobacter jejuni* - This is a microaerophilic organism that causes more than 2 million cases of gastroenteritis in the U.S.A.. Campylobacter species associated with diarrhoea belong most commonly, to the species *C. jejuni* or *C. coli.* Other less common pathogens in humans are *C. fetus* and *C. laridis.* Proctolitis in homosexual men has also been associated with *C. cinaedi* and *C. fennelliae* (Quinn et al., 1984). Commonly implicated as sources are raw milk, poultry and beef. The illness is characterized by moderate to severe watery diarrhoea (sometimes with blood and pus), nausea, vomiting and fever. Antimicrobial treatment is usually not needed. Erythromycin, tetracycline or aminoglycosides can be used in severe cases when blood cultures are positive.

1.3.3. *Shigella* - Shigellosis is caused by *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), or *S. sonnei* (subgroup D). *S. flexneri* and *S. sonnei* are isolated more often than the other two species; *S. sonnei* is the most common cause of the illness in the U.S.A. and *S. flexneri* in Mexico (Radetsky, 1986).

The illness is acquired by ingestion of food or water contaminated by excreta of infected patients. Voluminous watery stools during the first 24-48 hours are followed by bloody mucoid stools, faecal urgency and tenesmus. Most patients recover with symptomatic treatment and oral rehydration. Severely ill patients may require parenteral rehydration and antibacterial therapy with ampicillin, trimethoprim-sulphamethoxazole, or fluoroquinolones. Because *S. sonnei* is commonly resistant to ampicillin and TMP-SX, determination of its susceptibility by *in vitro* testing is necessary.

1.3.4. *Escherichia coli* - These organisms are found in large numbers as part of the normal flora, but certain strains are associated with diarrhoeal disease. These strains can be divided into four major groups: enterotoxigenic, enteropathogenic, enteroinvasive, and enterohaemorrhagic. A fifth strain enteroadherent is less well defined.

Enterotoxigenic *Escherichia coli* is a major cause of diarrhoea in infants in developing countries and is responsible for almost 40% of all travellers diarrhoea (Black et

al., 1982). It produces a heat-labile and a heat-stable enterotoxin.

Enteropathogenic strains are associated with epidemic-type diarrhoea in neonates and infants under 6 months of age. They produce distinctive lesions in the intestine by destroying the microvilli. Enteroinvasive infection causes a dysenteric form of disease and enterohaemorrhagic type illness is caused by a distinct serotype, O157:H7.

Enteroaggregative *Escherichia coli* (EAggEC) constitute the most recently described category of diarrhoeagenic *Escherichia coli*. EAggEC have been associated with small outbreaks of diarrhoea in children and adults in Great Britain (Scotland et al., 1991). Some EAggEC strains produce a novel heat-stable toxin which has been named EAST1. The enterotoxic effect of EAST1 appears to be mediated by guanylate cyclase stimulation (Savarino et al., 1991).

1.3.5. *Clostridium difficile* - *C. difficile* is a spore-forming, Gram positive, obligate anaerobic bacillus. It forms part of the normal intestinal flora in 3% of healthy adults. *C. difficile* is the most frequently identified enteric pathogen in patients with antibiotic-associated diarrhoea and colitis. It accounts for 10%-25% of all cases of antibiotic-associated diarrhoea and virtually all cases of antibiotic-associated pseudomembraneous colitis (PMC). Clinical features that distinguish infection with *C. difficile* are hyperpyrexia, leukemoid reactions, toxic megacolon, PMC and chronic diarrhoea. Factors important in the pathogenesis of disease are exposure to antibiotics, presence of *C. difficile* in the indigenous flora, production

of toxin A, and age-related susceptibility. The criterion standard for testing is the tissue culture assay; alternatives are culture and antigen detection methods such as EIA, dot blot, and latex agglutination. The main problems associated with *C. difficile* infection are the management and prevention of nosocomial epidemics, and the management of repeated relapses.

1.4. Enteric Parasites

The intestinal parasites include eukaryotic protozoan (unicellular) organisms and metazoan (multicellular) organisms (i.e. helminths or worms). *Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum,* and *Blastocystis hominis* are the more common protozoan parasites. *Dientamoeba fragilis, Balantidium coli, Isospora belli and Isospora hominis* are rarely encountered in developed countries and are mostly found in the tropics.

1.4.1. *Giardia lamblia* - is the most common of the protozoan parasites and occurs worldwide. Infection is acquired by ingesting food or water containing cysts. After an incubation of at least one week, the patient develops diarrhoea, foul-smelling stools, flatulence cramps and weight loss. Diagnosis can be confirmed by finding cysts or sometimes trophozoites in the patients stool. It is usually treated with metronidazole or tinidazole.

1.4.2. *Entamoeba histolytica* - The vast majority of infected persons are asymptomatic (85-95%). When disease occurs, it may take one of five clinical

forms: acute ulcerative proctocolitis, fulminating colitis, chronic colitis, typhloappendicitis, or amoeboma. Amoebic typhloappendicitis is characterized by a gradual onset of diarrhoea. It derives its name from the fact that the accompanying abdominal pain is localized in the caecum and caecal appendix. Amoeboma is an inflammatory swelling of the mucosa and /or sub-mucosa of the colon, with accompanying colitis. Laboratory diagnosis is made by observing trophozoites in diarrhoeal discharge or cysts in formed stool. Microscopic examination also shows Charcot-Leyden crystals but no leukocytes. Metronidazole is the current drug of choice (Ravdin 1986).

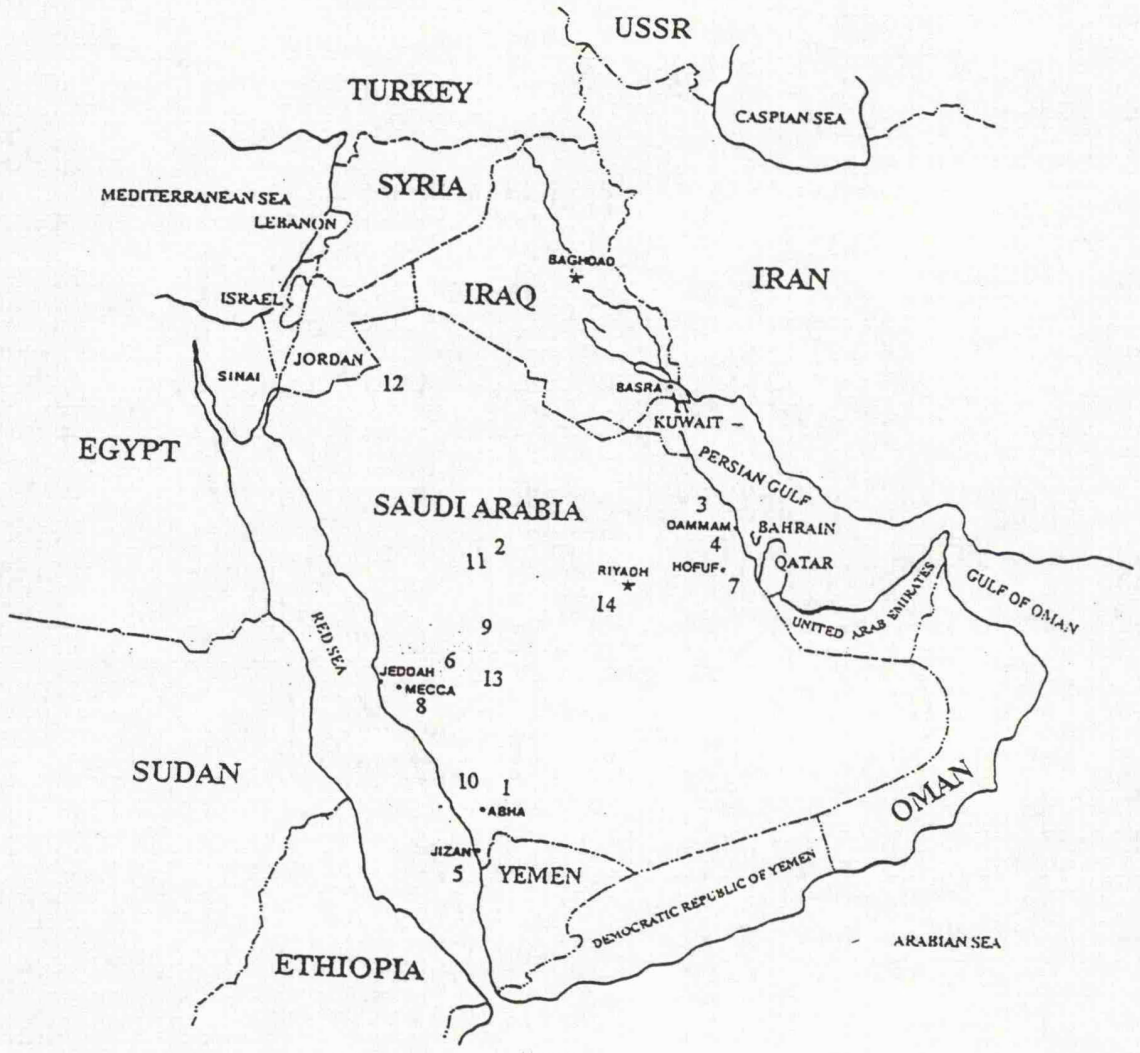
1.4.3. *Cryptosporidium parvum* - In immunocompetent patients, diarrhoea is mild and transient, with green,watery, offensive stools; sometimes abdominal pain and vomiting. Stools may contain mucus but no leukocytes. In immunodeficient patients, especially those with AIDS, diarrhoea is severe and persistent, lasting for months. Fluid loss in such cases may be as high as 12-17 L/day. Laboratory diagnosis is made by detecting oocytes in the patients stool (Connolly et al., 1988).

1.4.4. *Blastocystis hominis* - Although the prevalence of *B. hominis* infection ranges from 5-15% in different parts of the world, there is still doubt over its ability to cause enteritis. Reports suggest that it should be considered a pathogen in patients with recurrent symptoms, especially when a large number of cysts but no other pathogens are present in the stools (Sheehan et al., 1986).

1.4.5. Metazoa - These parasites include roundworms (*Ascaris lumbricoides*); pinworms (*Enterobius vermicularis*); whipworm (*Trichuris trichuria*) and hookworm (*Ancyclostoma duodenale*); tapeworms (*Taenia saginata* and *Taenia solium*) and flukes (*Schistosoma* species). Although metazoan parasites are important from an epidemiological point of view, as diarrhoeal agents they are less significant than bacteria, viruses and protozoa. Most often they cause either no symptoms or only mild to moderate diarrhoea, with abdominal discomfort nausea and vomiting.

Tropical and sub-tropical regions support a diverse group of parasites. Warm temperatures permit survival and maturation of these organisms into infectious forms. Poor sanitation and hygiene, low standards of living and education, close contact between persons living at a subsistence level favour the spread of parasites. Human infection typically follows ingestion of contaminated food or water, or invasion of larva through exposed skin (Jernigan et al., 1994). For example, the prevalence rate of parasites in Laos ranges from 77% to 96% depending on living conditions. This is due to the habit of eating raw flesh and intimate contact with domestic animals (Giboda et al., 1991).

FIG. 2.11. Map of Saudi Arabia and the Middle East.



Key to : Medical Centres in Saudi Arabia

1. Abha general hospital - **Abha**
2. King Fahad Specialist Hospital - **Buraidah**
3. Dammam Central Hospital - **Dammam**
4. Dhahran medical center - **Dhahran**
5. King Fahad Central Hospital - **Gizan**
6. Central hospital - **Jeddah**
   King Abdulaziz general hospital - **Jeddah**
   King Abdulaziz University hospital - **Jeddah**
7. King Fahad University hospital - **Al-Khobar**
8. Al-Noor Specialist hospital - **Mecca.**
9. King Abdulaziz hospital - **Medina**
   King Fahad Hospital - **Medina**
10. Najran general hospital - **Najran**
11. Oneizah general hospital - **Oneizah**
12. King Fahad Hospital - **Tabuk**
13. King Faisal hospital - **Taif**
14. King Faisal Specialist Hospital and Research Centre - **Riyadh**
    Riyadh Central hospital - **Riyadh**

Chapter 2. Epidemiology of Enteric Infections in Saudi Arabia.

2.1. Aetiology of Gastroenteritis at a Major Referral Centre in Saudi Arabia.

Introduction

In order to estimate the aetiology of gastroenteritis at a major referral center in Saudi Arabia. A retrospective study was carried out on 58,110 fresh stools from 40,338 patients. This work was carried out routinely on a daily basis in the Microbiology department by technical staff. The stored data was used in this analysis. Examination for bacterial, viral, protozoan or metazoan parasites was based on the clinical judgement of the attending physician. Therefore, all specimens were not processed for the presence of all organisms.

Materials and Methods.

This study was conducted at King Faisal Specialist Hospital and Research Centre, a 550-bed tertiary care centre in Riyadh, which serves as a referral facility for Saudi Arabia and the Middle East. The admissions in the hospital average over 13,000 and outpatient visits approximately 350,000 per year. A total of 58,110 fresh stools from 40,338 patients were examined for the presence of viral, bacterial and intestinal parasites. Some of these specimens were from patients that had been

referred to this facility from other centres in Saudi Arabia (Fig. 2.1.1.). Gastroenteritis suspected by clinicians was based on any combination of the following signs and symptoms: nausea, vomiting, abdominal pain, tenesmus, acute or chronic diarrhoea, distension, flatulence, dehydration, fever, seizures, meningismus, and blood or mucus in stools.

Stools for bacteria were received in the laboratory in wide-mouthed, water tight, plastic containers. The specimens were examined grossly for stool consistency and inoculated onto plates of 5%(v/v) sheep blood agar (BAP), MacConkey agar, Hektoen agar, Campylobacter BAP, and selenite broth. The Campylobacter plates were incubated at 42°C in a microaerophilic jar with an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen for 48 hours. All the plated media were incubated at 35°C for 18-24 hours. Selenite broth was subcultured at 12 hours onto a MacConkey and a Hektoen agar plate and incubated for 18 hours at 35°C. All media were purchased commercially (Saudi Prepared Media, Riyadh, S.A. and can also be obtained from Gibco BRL, Uxbridge, UK) (appendix 3.1).

The enteropathogens were identified using API 20E (Analytab Products, Plainview, NY, US) and/or conventional methods which involve biochemical testing. (appendix 3.2). Using conventional biochemical methods, a primary battery of tests, including adonitol fermentation, arginine dihyrolase, citrate utilization, DNAase, gas from glucose, hydrogen sulphide, indole production, lysine decarboxylase, motility, ornithine decarboxylase, phenylalanine deaminase, sucrose fermentation, urease, and Voges-

Proskauer, were sufficient for identification of most isolates to the species level. A supplementary battery of tests were used to identify more difficult isolates and included carbohydrate fermentation tests plus tests for the hydrolysis of ortho-nitrophenyl-galactoside (ONPG) and for growth in KCN.

In the API 20E system,a number of tests contained on a plastic strip were inoculated with a suspension of the test organism, and after 24h incubation the results were combined to produce a 7-digit number which was looked up in a large database to determine the identity of the organism. Serogrouping was performed by using antisera from Wellcome Diagnostics (Dartford, England) and BBL (Cockeyville, Maryland, USA), as per manufacturer's directions. All diarrhoeal specimens were also examined microscopically following methylene blue staining, and occult blood analysis was performed using Haemocult slides (SKD, San Jose, Ca.,USA).

Faecal specimens for intestinal parasites were grossly examined for stool consistency. Watery or acute diarrhoeal specimens were examined microscopically within 60 minutes of their receipt in the laboratory using saline and iodine-stained wet mount preparations. Soft or formed stools were examined by these procedures within 6 hours of receipt, or placed in sodium-acetate-acetic acid formalin (SAF) for preservation. All specimens were subsequently concentrated by the ethyl acetate-formalin method (Young et al., 1979). Haematoxylin stain was also performed on all liquid specimens and those in which identification of parasite(s) was difficult or equivocal.

To detect rotaviral antigens in faecal specimens an enzymeimmunoassay (EIA) was performed with the Rotazyme II kit (Abbott Diagnostics, Irvine, Tx.). This test employs polystyrene beads that are coated with guinea-pig antirotavirus antibodies. The Rotazyme II kit has been found to be 99.4% sensitive and 97.3% specific with an overall agreement of 98.7% when compared with electron microscopy. Positive and negative controls were tested with all batches of patient specimens.

Stools for electron microscopy (EM) were filtered and the supernatant centrifuged at 37,400 x g for 1 hour at 4°C in a refrigerated Beckman L7-55 ultra-centrifuge (Palo Alto, Ca., USA). Formvar/carbon grids (200 mesh) were prepared using negative staining and examined on a Jeol electron microscope (Tokyo, Japan). Adenovirus was also detected by EIA, which used a group specific antibody including type 40/41. Testing was carried out according to manufacturer's instructions (IDEIA, Dako, Cambridge, UK).

Clostridium difficile toxin was tested by using the cytotoxic assay. Stool filtrates were prepared by diluting the specimen 1:5 with tryptose phosphate broth with 0.5% gelatin, gentamicin, and amphotericin B (Flow Labs, Irvine, Scotland), and centrifuged at 1900 x g for 10 minutes. The supernate was filtered through a 0.45 μm low protein binding filter and 0.1 ml inoculated into two tubes of diploid fibroblast (human foreskin) cell cultures. A third tube was used for neutralization and received 0.1 ml of supernate and 0.1 ml of *Clostridium sordelli* antitoxin (Biologics, FDA,Bethesda,MD). The supernatant from a toxigenic strain of *C.*

difficile was used as a positive control. The cell cultures were incubated at 35°C and examined for cytotoxic effects at 24 and 48 hours.

Results

Bacterial enteropathogens were sought in 43,178 stools from 27,820 patients. These were isolated from 2,133 (7.7%) patients, with *Salmonella* being the most frequent (51.7%), followed by *Campylobacter* (28%), *Shigella* (14.9%), and *Aeromonas hydrophila* (4.6%) (Table 2.1.2.). Bloody diarrhoea was more common in patients with shigellosis (32%) than those infected with other bacteria. Stool specimens from 80% of the patients with *Shigella* gastroenteritis had leukocytes, compared with approximately 40% of patients with *Salmonella* or *Campylobacter*. In patients with *Salmonella* infections approximately 60% had symptoms of fever, vomiting and dehydration; vomiting occurred in patients with shigellosis (40%). Most of the species of salmonellae were *S. enteritidis* and *S. cholerasuis*

Stool specimens from 3,817 patients were examined for intestinal parasites. Protozoan or metazoan parasites were found in 1,063 (27.8%) patients, yielding a total of 1,411 organisms. The incidence of potentially pathogenic parasites was 22.9% of the total and commensal was 77.1%. Of the first group, *Giardia lamblia* was most common, followed by *Hymenolopsis nana*. Among the commensals, *Entamoeba coli* was most commonly found, followed by *Endolimax nana* and *Blastocystis hominis* (Table 2.1.3.).

A total of 7,439 stool specimens from 6,842 patients were tested for rotavirus. Patients with positive stools numbered 965(14.1%) with 70% of infections in children under 5 years of age. Electron microscopy and EIA were also used to examine 1200 acute diarrhoeal specimens for adenoviruses. These were seen in 64(5.3%) patients, 15(1.3%) had small round viruses and 4(0.3%) coronavirus Table 2.1.4.). Of the 64 patients with adenovirus infection 35 were in children under 5 years of age. There were 734 males and 566 female patients.

Presence of *Clostridium difficile* toxin was tested in 2,356 patients from a total of 2,476 specimens. Of these there were 224 (9.5%) positive. 158 (70%) of these patients were organ transplant, bone marrow transplant recipients or neutropenic patients who were given immunosuppressive therapies including one or more of the following antimicrobial agents: metronidazole, vancomycin, sulphamethoxazole, ticarcillin, amikacin, ampicillin, cefazolin, imipenem, ceftazidime, trimethoprim, amphotericin B, gentamicin and fluconazole.

Table 2.1.2. Distribution of bacterial enteropathogens which were found in stool when samples from 27,820 patients with suspected gastroenteritis were examined.

Bacterial group or species	No. positive patients (%)
Salmonella	
Serogroup A (not paratyphoid A)	3 (0.1)
Serogroup B (not paratyphoid B)	429 (20.1)
Serogroup C (not paratyphoid C)	435 (20.4)
Serogroup D (not paratyphoid D)	82 (3.8)
Serogroup other than A-D	153 (7.2)
Campylobacter jejuni	597 (28.0)
Shigella	
Group A	4 (0.2)
Group B	140 (6.6)
Group C	259 (1.2)
Group D	148 (6.9)
Aeromonas hydrophila	98 (4.6)
Vibrio cholerae, V. flavis	19 (0.9)

Table 2.1.3. Species distribution of intestinal parasites which were found in stool samples from 1063 patients when samples from 3817 patients with suspected gastroenteritis were examined. (Data extracted from routine laboratory reports).

Parasite	No. patients positive (%)
Protozoan pathogen :	
Giardia lamblia	196 (13.9)
Entamoeba histolytica	24 (1.7)
Dientamoeba fragilis	3 (0.2)
Metazoan Pathogen :	
Hymenolopsis nana	32 (2.3)
Schistosoma mansoni	24 (1.7)
Trichuris trichiura	18 (1.3)
Ascaris lumbricoides	14 (1.0)
Dicrocoelium dendriticum	7 (0.5)
Taenia sp.	4 (0.3)
Schistosoma haematobium	1 (0.1)
Commensals :	
Entamoeba coli	414 (29.3)
Endolimax nana	297 (21.0)
Blastocystis hominis	187 (13.2)
Chilomastix mesnili	85 (6.1)
Iodamoeba butschlii	57 (4.1)
Entamoeba hartmanni	45 (3.1)
Trichomonas hominis	3 (0.2)

Total = 1411(100%)

Table 2.1.4. Distribution of viral agents of gastroenteritis in stool samples from patients with suspected gastroenteritis.

Virus	No. of patients	No. of patients positive (%)
Rotavirus	6842	965 (14.1)
Adenovirus *	1200	64 (5.3)
Small round viruses (SRVs)*	1200	15 (1.3)
Coronavirus*	1200	4 (0.3)

***Age group of 1200 patients at K.F.S.H. & R.C.:**

Age group	Number
Under 5	416
6 - 19	238
20 -55	413
Over 55	134

Discussion

In developing countries bacteria account for 25-40% of diarrhoea in Mexico (Olarte et al., 1988), 20% in Thailand (Echeverria et al., 1985), 19% in Central Africa (Georges et al., 1984), 16% in Brazil (Guerrant et al., 1983), and 14% in Ethiopia (Ashenafi et al., 1985), compared to 5-10% in the U.S.A. (Guerrant et al., 1986; Jones and Rodriguez, 1988). In Ethiopia, Thailand, Brazil and Mexico, *Shigella* is the most common pathogen, with a prevalence rate of 8-12%. In Central Africa, *Campylobacter* predominates with a prevalence of 10.7%.

At this hospital bacterial aetiology was observed in 7.7% of specimens with *Salmonella* being the most frequent, followed by *Campylobacter* and *Shigella.* Another report on Saudi chidren (Al-Bwardy et al., 1988) also found *Salmonella* to be the most common bacterial pathogen, followed by *Campylobacter.*

The prevalence of protozoan and metazoan parasites varies considerably in different populations but is generally considered to be high in tropical and sub tropical countries. Of the protozoan parasites implicated in clinical disease, only *G. lamblia, E. histolytica, D. fragilis, B. hominis*, and *Cryptosporidium parvum* were detected at this facility. Incidence of *G. lamblia* in stool specimens in previous studies in Riyadh has ranged between 9-36% (Mahgoub et al., 1980; Al-Saud et al., 1983; Bolhol et al., 1984; Qadri and Khalil, 1987) of all the pathogens, whereas in our patient population it was found to be approximately 14%.

In industrialized countries, rotaviruses have been shown to be the most common enteropathogens, accounting for over 50% of such infections in infants and children (Kapikian et al., 1976, Birch et al., 1977; Jones and Rodriguez, 1988). Their prevalence is lower in developing countries: 20-30% in Mexico, 19% in Brazil, and 18% in Central Africa. The occurrence of rotavirus has been reported to vary between 10-44% in the Saudi population (Walker et al., 1984; Arya et al., 1985; Jamjoom et al., 1986; Al-Bwardy et al., 1988; Al-Ahdal et al., 1991; Akhter and Qadri, 1992). The prevalence of rotavirus in this study was found to be 14% in which 70% of infections were in children less than 5 years of age. There was no seasonal variation noted.

Adenoviruses are a frequent cause of viral diarrhoea, second only to rotaviruses (Uhnoo et al., 1986). The prevalence at this hospital was found to be 5%. There have been no other reports of adenovirus gastroenteritis in Saudi Arabia, hence further work is required to determine its true prevalence and epidemiology.

The prevalence and aetiology of microbial infections in this study are more akin to that seen in the U.S.A. and Europe than that found in tropical and subtropical countries. This may be due to the fact that the patients at this facility belong to a higher socio-economic group and Saudi Arabia due to its wealth has a well developed infrastructure. Epidemiological studies on the aetiology of gastroenteritis have paved the way towards understanding the causes and nature of these ubiquitous agents. Further studies are necessary to understand the trends and patterns of these infectious organisms for better patient management and knowledge of indigenous aetiologies.

2.2. Changing Patterns of Antibacterial Resistance in Clinical Isolates of *Enterobacteriaceae* at a Tertiary Care Center in Saudi Arabia.

Introduction.

Diarrhoeal disease and other infections due to enteric bacterial pathogens represent a critical medical problem in large areas of the world. Members of the *Enterobacteriaceae* play a major role in everyday clinical practice, especially bacteraemia, urinary tract infections, neonatal sepsis and meningitis and respiratory infections. It has been estimated that in the clinical laboratory half the septicaemia isolates, two-thirds of the bacterial gastroenteritis isolates, and three-quarters of the urinary tract isolates are accounted for by the *Enterobacteriaceae* (Kelly et al., 1985). Dilution tests are used to determine the minimal concentration of an antimicrobial agent required to inhibit or kill a microorganism. Serial dilutions of the antimicrobial agent are inoculated with the organism and incubated. The MIC is the lowest concentration without visible growth. The terms broth (or tube) and agar (or plate) are added to the term dilution test to indicate tests performed in liquid and agar media, respectively.

A significant problem to emerge has been that of antimicrobial resistance and the alarming frequency of multi-resistant strains of bacterial pathogens that have occurred in less affluent areas of the world. Much of the blame for this is attributed to countries where ready availability of antimicrobial agents has promoted heavy and indiscriminate usage of these drugs. Tertiary care facilities

are implicated due to intensive use of drugs and nature of patient population (Farrar,1985).

Large outbreaks have involved many of the important aetiologic agents of bacterial diarrhoea. In Mexico, an outbreak of dysentery due to *S. dysenteriae* type 1 which began in 1968 rapidly spread to involve six Central American countries. Morbidity and mortality were very severe since it was resistant to sulphonamides, streptomycin, tetracycline and chloramphenicol (Gangarosa et al., 1969).

Escherichia coli is an important cause of nosocomial and community acquired disease in everyday hospital practice. It can be associated with nursery outbreaks of diarrhoeal disease and travellers diarrhoea. Studies from the far east have shown that 72% are resistant to one or more antibiotics and 44% to at least four drugs (Echeverria et al., 1978). Studies on resistance of *Escherichia coli* to trimethoprim-sulphamethoxazole (TMP-SX) from strains collected in Chile, Thailand, Honduras, Costa Rica and Brazil have shown resistant strains in 38-50%. However, resistance to TMP-SX has only been found in 4-6% of isolates at medical centers in the U.S.A. (Murray et al., 1985).

Salmonella species are the aetiological agents of most of the bacterial food-borne disease in the United States. Over 50,000 cases were reported to the Centers for Disease Control (CDC) in1987. Chloramphenicol resistant strains of *S. typhi* have been isolated from India and South East Asia (Paniker and Vimala, 1972; Rowe and Threlfall, 1984). Outbreaks due to multi-resistant *Salmonella* other than *S.*

typhi and also to *Shigella* because of their widespread use have become more common. (Shahid et al., 1985; Macaden and Bhatt, 1985).

Particular problems arise from the fact that infections with multi-resistant micro-organisms often occur in the most critically ill patients. In the hospital setting, worrying resistance traits to emerge include plasmid-mediated resistance to imipenem and to third generation cephalosporins among nosocomial gram-negative bacteria (Murray, 1992).

This tertiary care facility treats many of the Kingdom's most seriously ill patients. In order to determine the changing pattern of antimicrobial resistance, data from six years was analyzed. Antimicrobial susceptibility testing was carried out routinely by the technical staff in the Microbiology Department and stored data analyzed.

Method.

Antimicrobial susceptibility of members of the *Enterobacteriaceae* that were isolated from clinical specimens between June 1988-March 1994 were analyzed. The bacterial isolates were identified by standard methods described in the Manual of Clinical Microbiology (Isenberg 1992). One isolate from each patient was tested to avoid the duplication of the same isolate. The bacteria analyzed consisted of 7592 *Escherichia coli*, 3182 *K. pneumoniae*, 387 *Klebsiella* sp., 996 *Proteus* sp., 296 *Morganella*, 803 *Enterobacter* sp., 322 *Citrobacter* sp., 600 *S. marcescens*, 1060 *Salmonella* sp., and 229 *Shigella* sp..

Disk-diffusion tests were performed using commercially prepared antimicrobial disks (Difco Laboratories, Detroit, Mich., USA). Antimicrobial susceptibility testing was performed according to the methodology described by the NCCLS. The medium used was Mueller-Hinton agar with a pH between 7.2 and 7.4. All incubations were at 35°C for 18-24 hours. Quality control (Q.C.) organisms used were *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All the Q.C. results were within acceptable ranges (Appendix 3.3).

Statistical Analysis.

The main objective of performing quantitative analysis was to present statistical evidence regarding increasing or decreasing patterns in the susceptibility of various bacterial pathogens over a specified period of time. The proportions of isolates susceptible were ranked for this period and Spearman's rank correlation was computed to check a significant increasing or decreasing trend. The value of the correlation coefficients r and p values gave an indication about the strength of relationship between time and susceptibility. A higher value of r (close to 1) was interpreted as an increasing trend and indicative of a positive correlation (ie. increased susceptibility). A value of r close to -1 exhibited a decreasing trend indicative of a negative correlation (ie. increased resistance). A p value less than 0.05 was considered significant as this indicates a very low error rate. A computer programme JMP from SAS institute was used to carry out the statistical analysis.

Results.

A total of 15,467 isolates from clinical specimens at KFSH & RC were evaluated to determine the susceptibility of enteric bacteria to routinely used antimicrobial agents. Antimicrobial susceptibility patterns of the isolates to 14 antibiotics are shown in (appendix 1). It was found that there was a significant increase in drug resistance in all the *Enterobacteriaceae* over the six years (Fig. 2.2.1 - 2.2.10.). *Escherichia coli* showed increased resistance to ampicillin from 60% in 1988 to 72% in 1994. There was also increased resistance to augmentin, cephazolin, netilmicin, piperacillin, trimethoprim-sulfamethoxazole (TMP-SX). There was no significant change in susceptibility to amikacin, cefoxitin, imipenem and ciprofloxacin (Fig. 2.2.1). *Salmonella* species exhibited decreased susceptibility to gentamicin, tetracycline, cephazolin, and TMP-SX (Fig 2.2.2.). *Enterobacter* species (Fig. 2.2.6.), *Citrobacter* species (Fig. 2.2.7.), *Serratia marcescens* (Fig. 2.2.8.), and *Proteus mirabilis* (Fig. 2.2.9.) showed marked fluctuations in susceptibility patterns over certain years. This may be due to the changing nature of the expatriate population and also that during these periods fewer isolates were tested.

Shigella sp. exhibited increased resistance to TMP-SX from 78% to 87% over the six years (Fig. 2.2.10.). *Klebsiella*, *Proteus* and *Citrobacter* sp. became more resistant to cephazolin, gentamicin and tetracycline. In particular, the susceptibility of *Klebsiella* sp. to gentamicin decreased from 94% to 61%. Despite increases and decreases in resistance patterns of many of the antimicrobials, some retained their

effectiveness. For, example, TMP-SX and amikacin retained or increased their activity to *Salmonella* over the six years. *Shigella* remained susceptible to ceftazidime, ampicillin, and cefoxitin. This may be due to the fact that antibiotic therapy is not usually indicated in these cases and that these agents were less widely used (Appendix 1).

From the statistical analysis all negative r values and p values less than 0.05 were considered as negative correlation and indicative of resistance. For example, in the case of *Escherichia coli*, statistical evidence for resistance was found in the case of ampicillin and nitrofurantoin. In the case of *Klebsiella sp.*, cephazolin, and ceftazidime were found to be statistically significant. All positive r values and p values less than 0.05 were indicative of increased susceptibility (Table 2.3.).

Antimicrobial Susceptibilities of *Enterobacteriaceae* at KFSH & RC.

Fig. 2.2.1. Eschericia coli

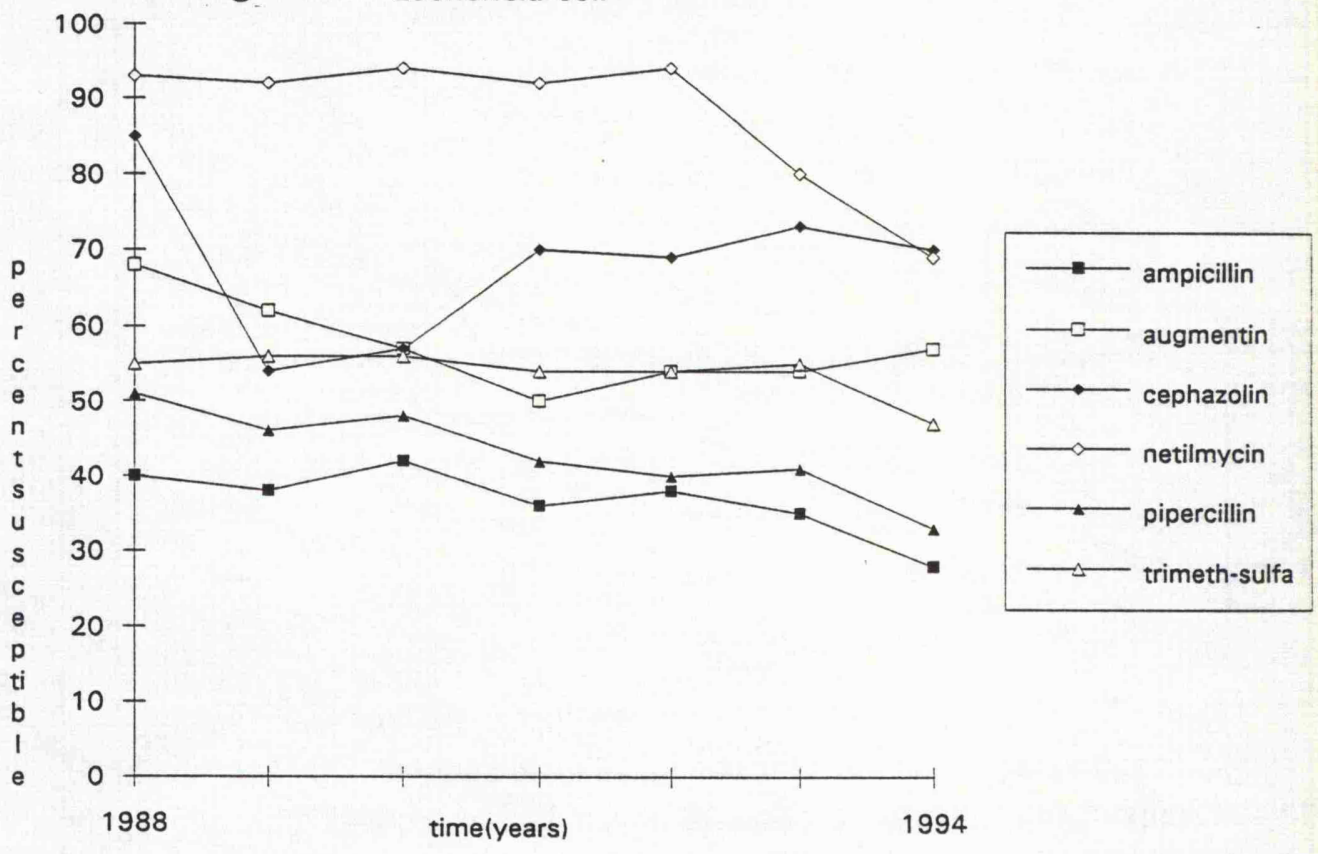


Fig. 2.2.2. Salmonella sp.

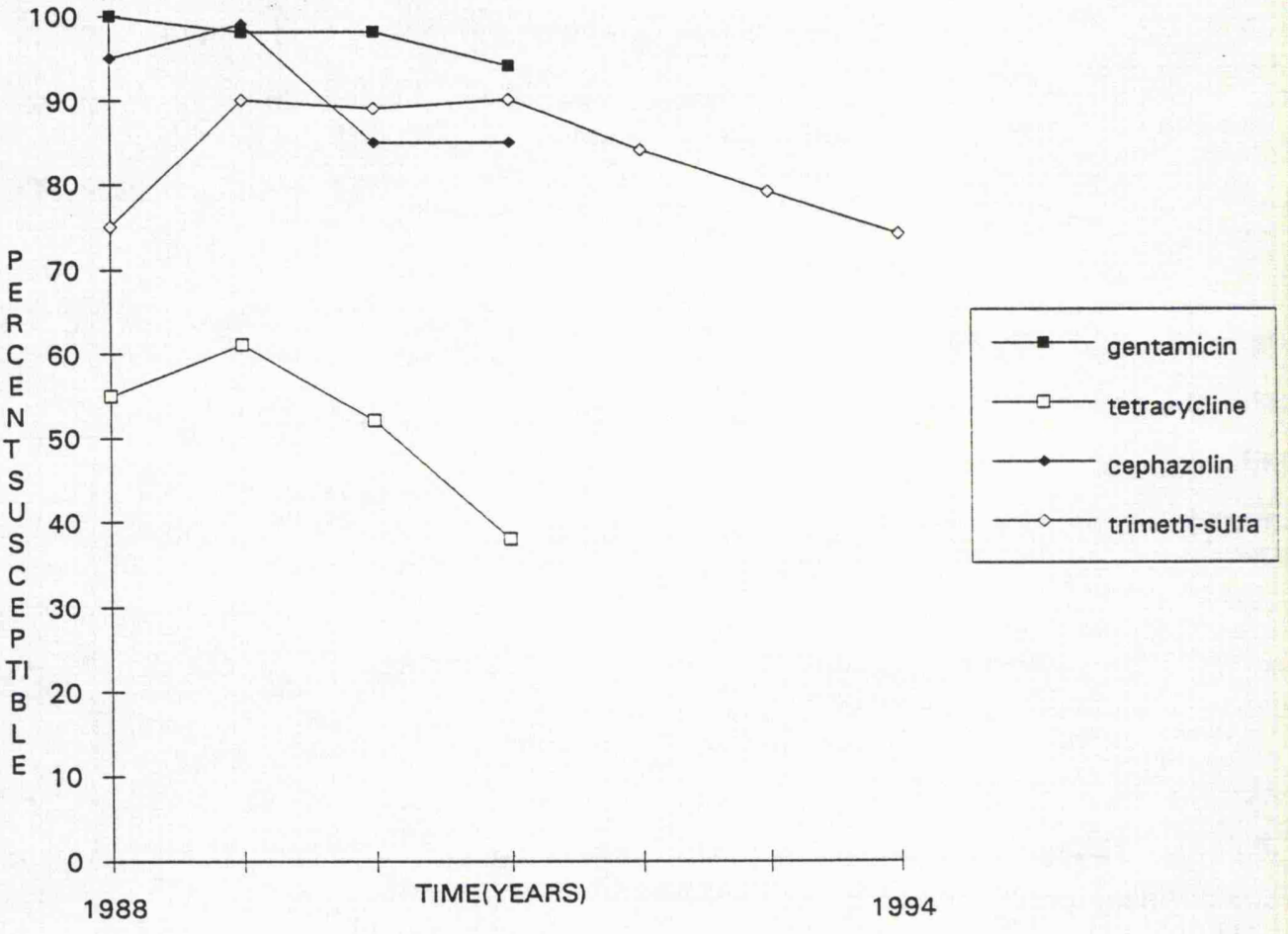


Fig. 2.2.3. Klebsiella sp.

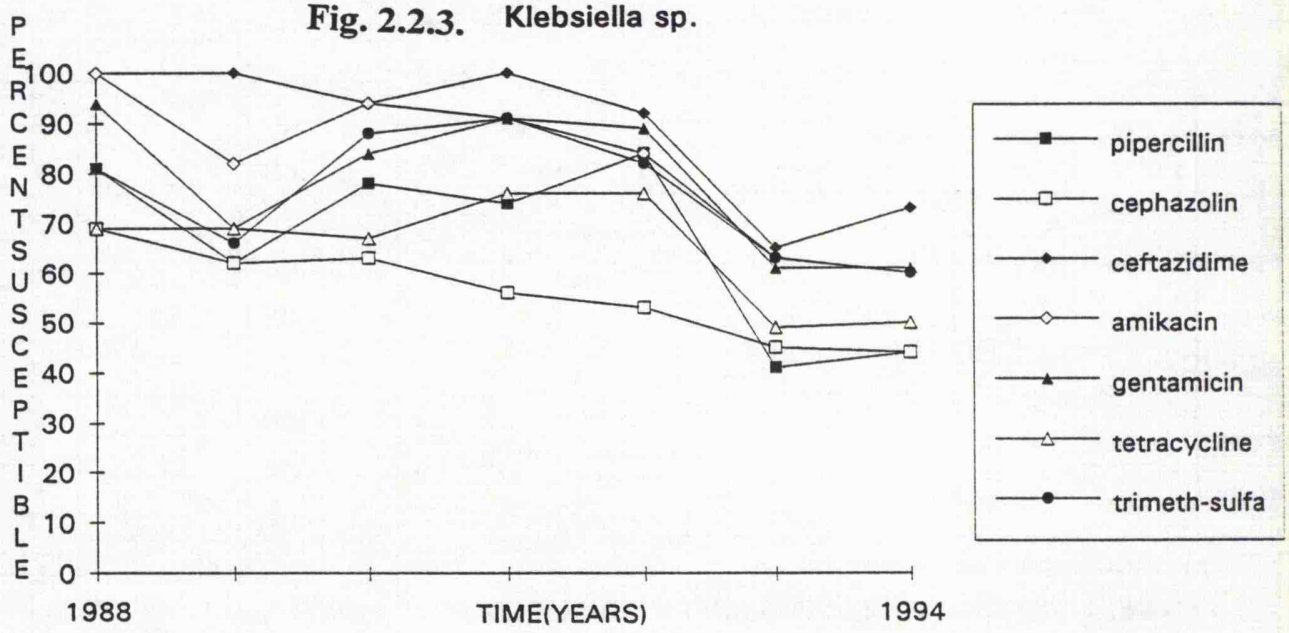


Fig. 2.2.4. **Klebsiella pneumoniae**

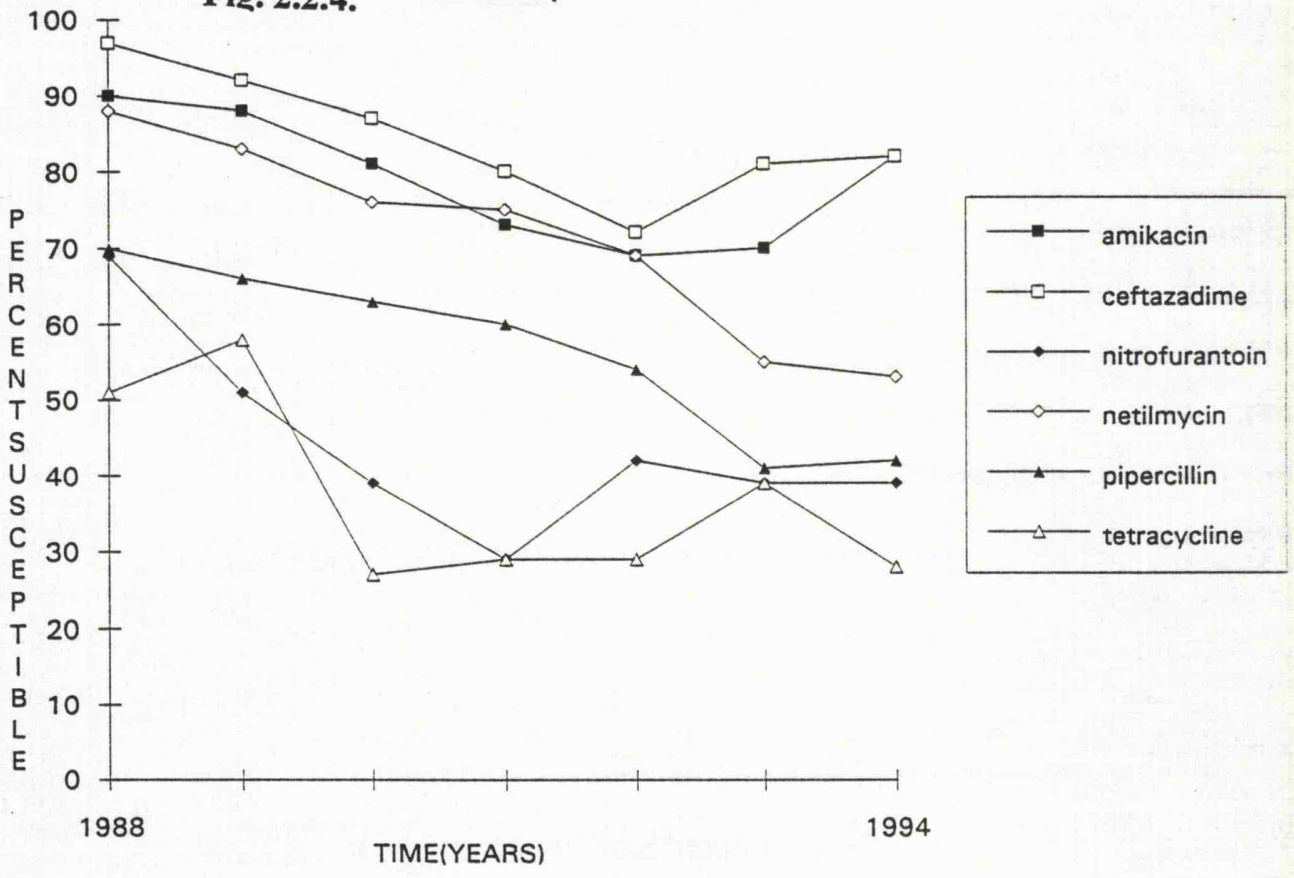


Fig. 2.2.5. Morganella sp.

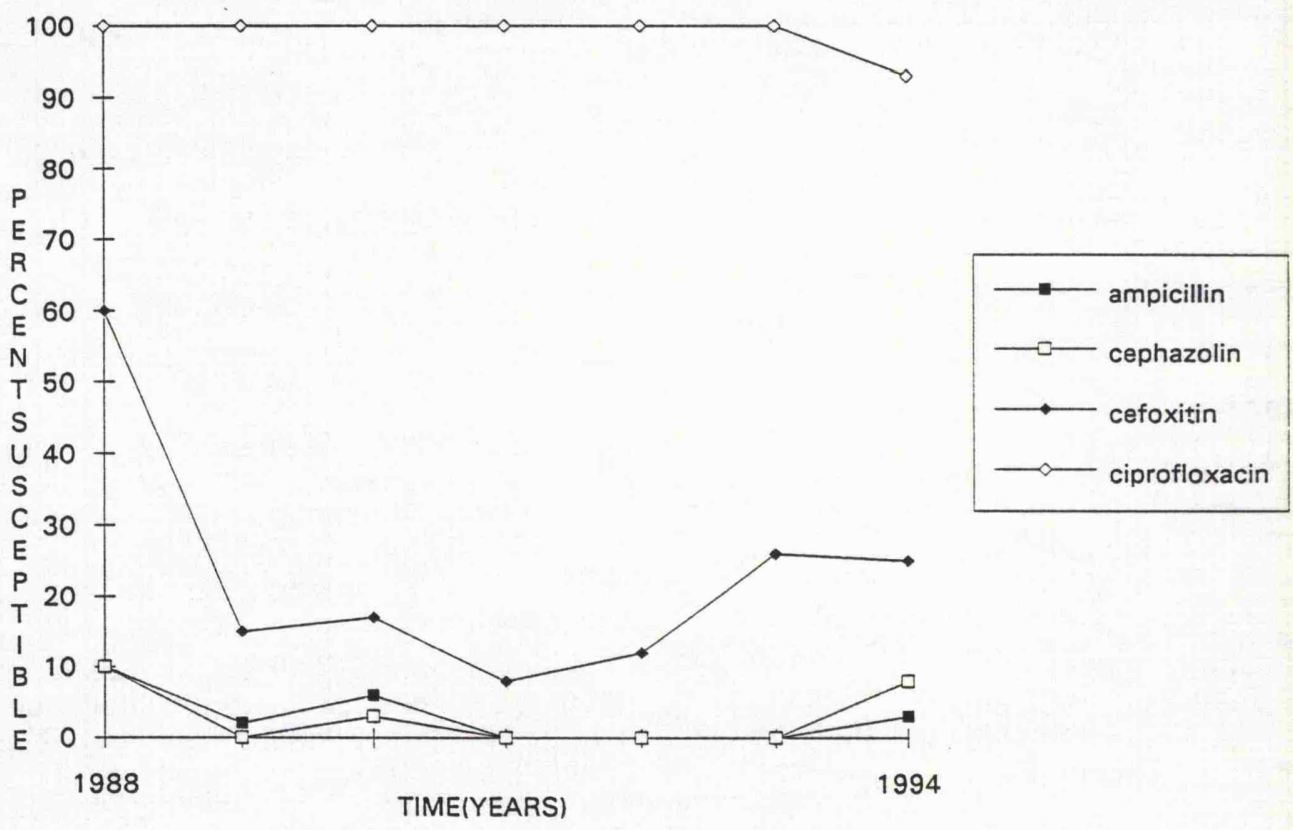


Fig. 2.2.6. Enterobacter sp.

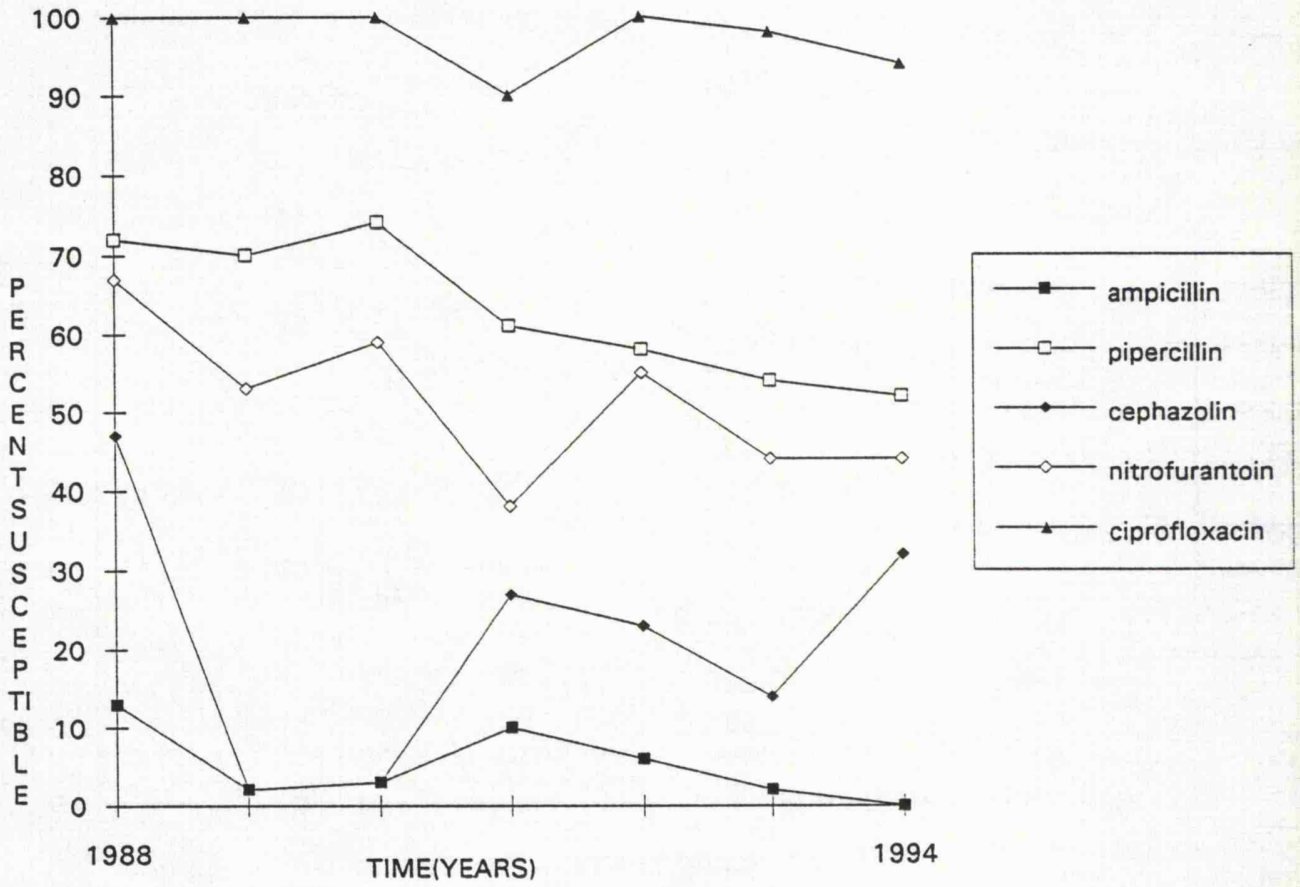


Fig. 2.2.7. Citrobacter sp.

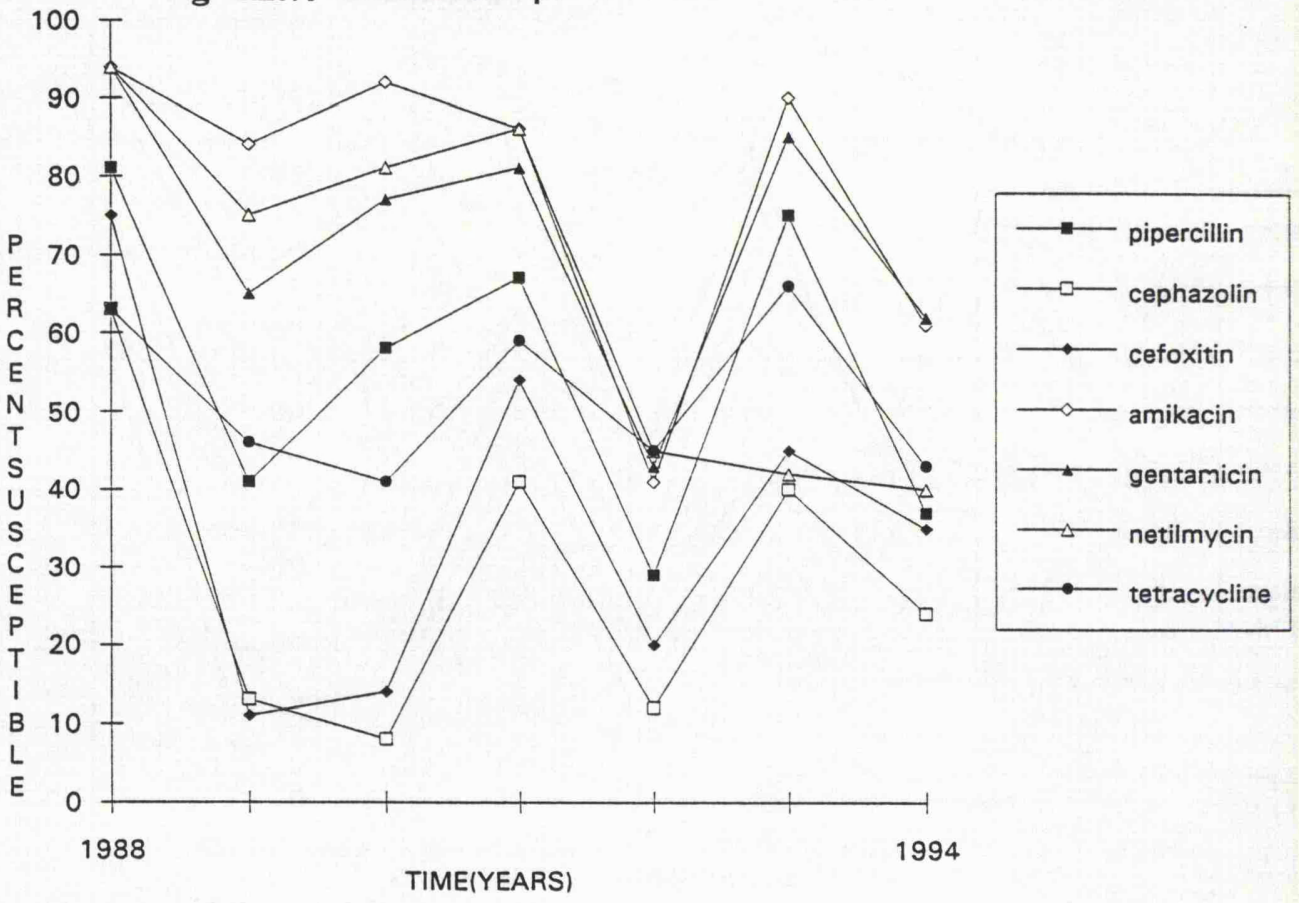


Fig. 2.2.8. Serratia marcescens

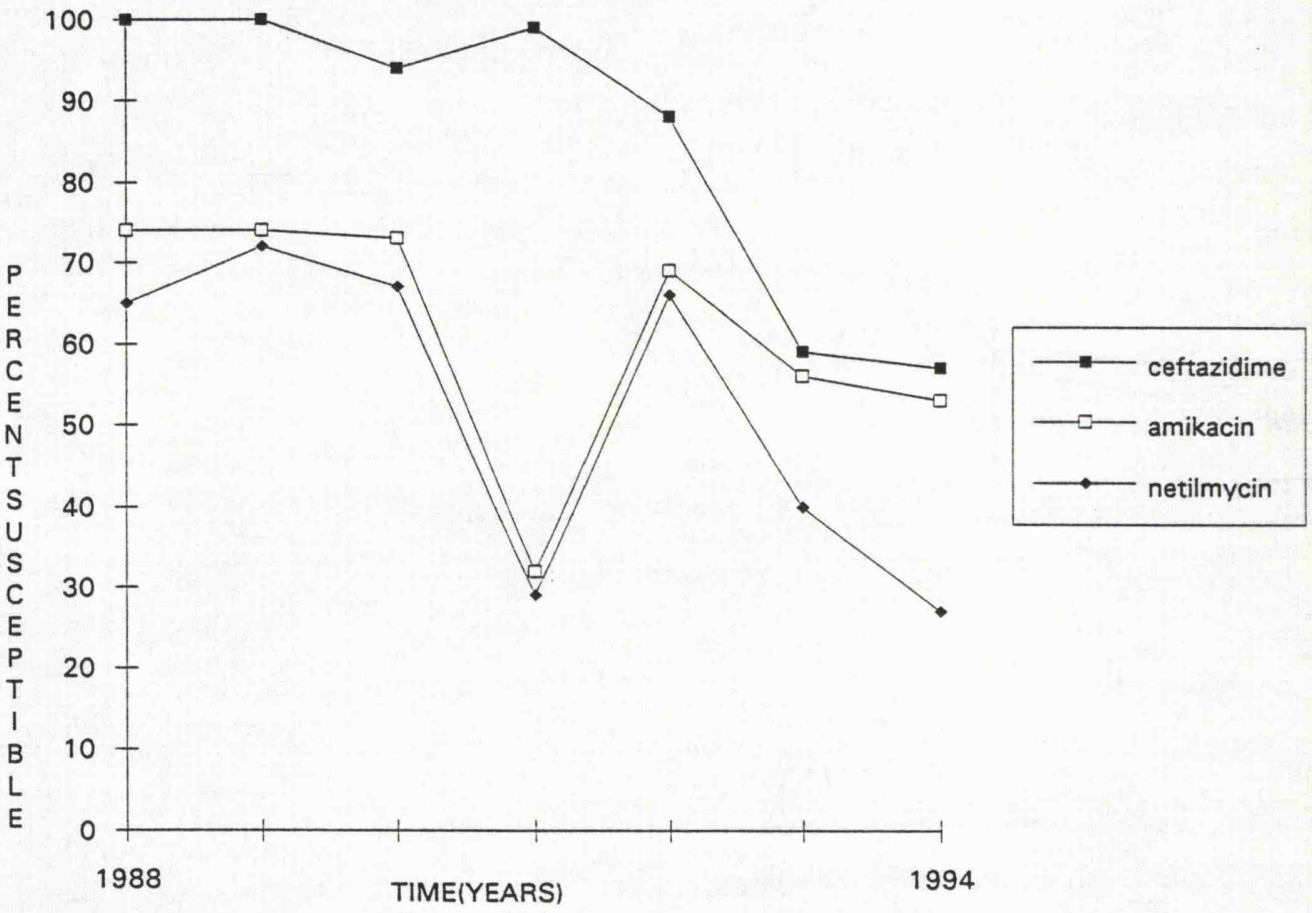


Fig. 2.2.9. Proteus mirabilis

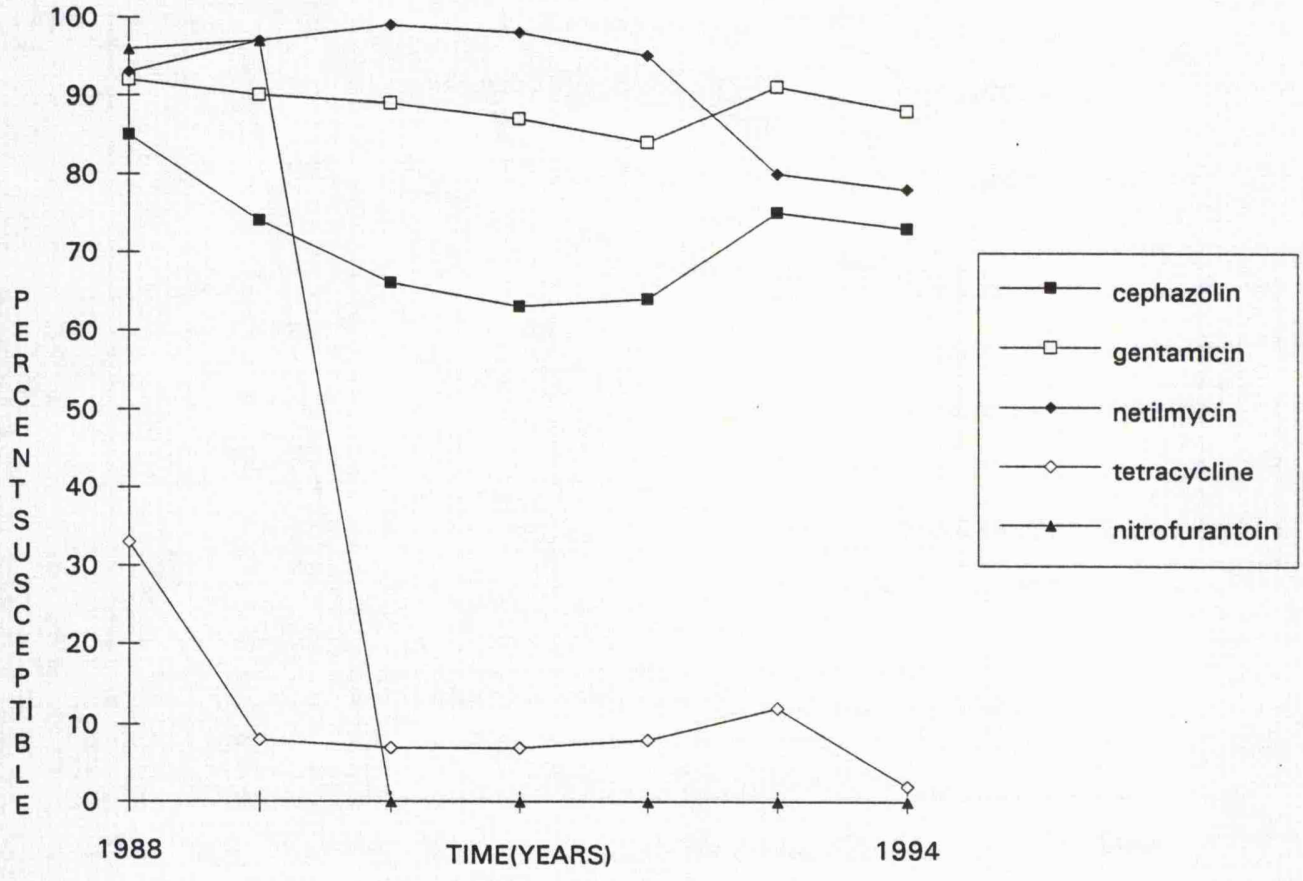


Fig. 2.2.10. Shigella sp.

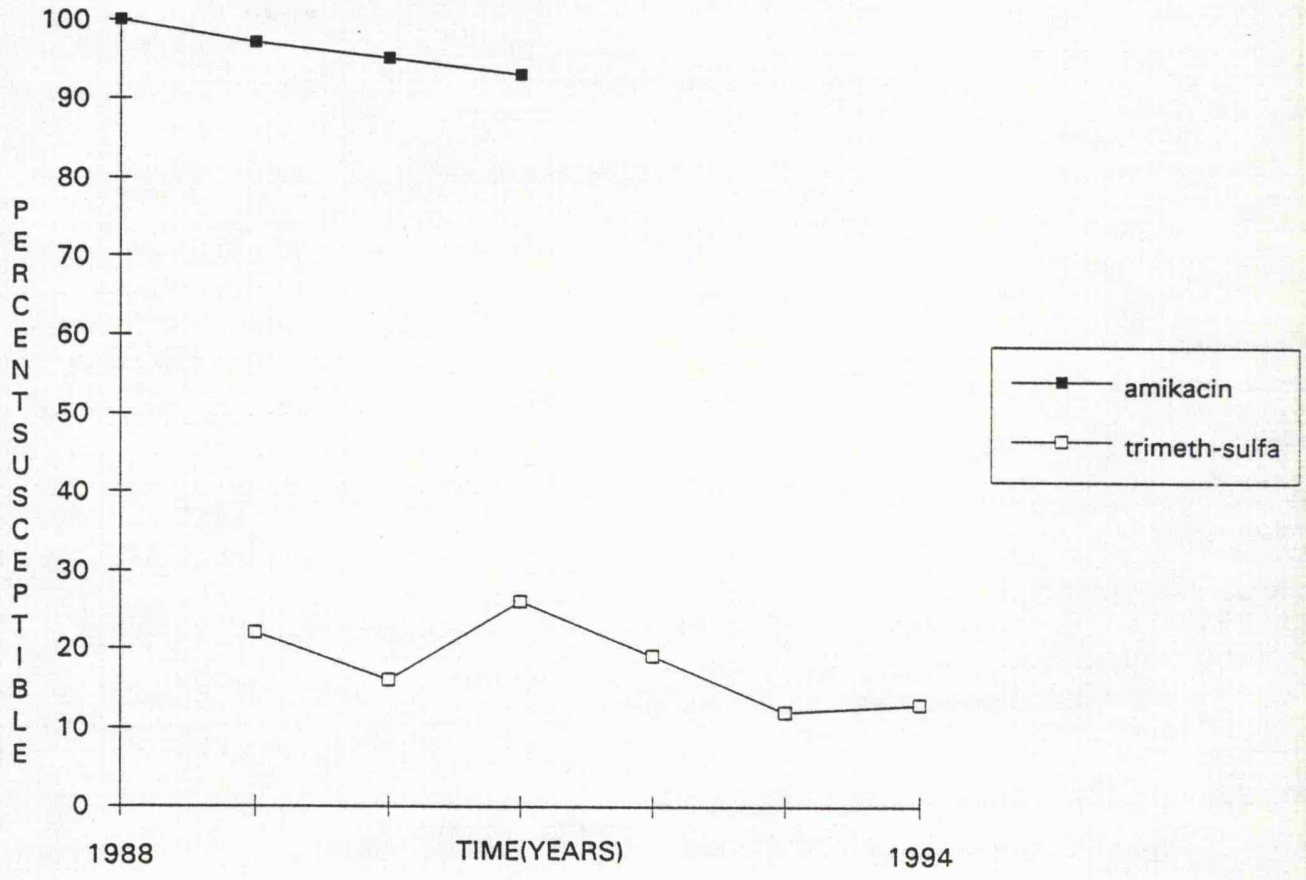


Table 2.3. Correlation Analysis of Antimicrobial Susceptibility Patterns Over Time.

Susceptibility	TIME													
	r values for each drug													
Organism	AM	AN	AUG	CF	CAZ	CFOX	FUR	GM	NET	PIP	TMP-SX	TE	IMP	CIP
E. coli	*-0.9	-0.9	-0.6	-0.2	-1	-	*-0.9	0.2	-0.2	-1	-0.8	-0.3	-	0.4
Morganella	-0.5	0	0.3	-0.2	0.66	-0.1	0.11	0.7	0.6	0.2	0.5	0.3	-	-0.8
Klebsiella sp.	-0.6	*-0.9	-0.56	*-1.0	*-0.9	-0.6	0.8	-0.7	-0.6	-0.8	-0.5	-0.6	-	-
K. pneumoniae	-0.4	-0.7	-0.7	-0.36	*-0.9	0.86	-0.8	0	*-1.0	*-1.0	-0.1	-0.7	-0.44	-0.4
Proteus	0.3	0.6	0.7	-0.6	-0.6	-0.2	-0.7	-0.89	-0.3	-0.05	0	-0.7	-0.2	*1.0
Citrobacter	-0.5	0.7	-0.6	-0.6	-0.3	-0.3	-0.7	-	0.1	-0.1	-0.4	-0.6	0.3	0
Salmonella	-0.7	-	-0.6	-	-0.4	-	-	*-1.0	-	-	-0.4	-0.5	-	-
Shigella	0.3	-1	0.5	*1.0	-	-	-	0.5	-	-	*-1.0	-0.6	-	-
Enterobacter	-0.7	-0.1	0.7	0	0	0.66	*-1.0	*0.97	-0.15	*-1.0	*0.97	-0.3	-	-0.8
S. marcescens	0.9	-0.7	0.9	-	*-0.9	0.8	0.77	*0.9	-0.5	0.82	0.87	0.11	-	-0.3

KEY :

1 * = p is less than 0.05 (ie significant values)

2 Each cell represents the correlation (r) of time vs. microbial susceptibility for each drug.

Discussion.

Concerned with the adverse effects of antimicrobial agents of the time almost 90 years ago, Paul Ehrlich proposed development of "magic bullets". He envisioned drugs that would eradicate microbial pathogens without any adverse effect to the human host. Development of penicillin for clinical use during World War II appeared to have made his dream come true. However, the early euphoria on the success in treatment of staphylococcal and streptococcal disease by penicillin was followed by cynicism with the development of penicillin-resistance in over 50% of hospital isolates by the mid 1950's. The same story has been repeated many times as new antimicrobials, whether penicillins, cephalosporins, aminoglycosides, quinolones or macrolides were introduced into clinical practice. Now emergence of resistance in the infecting organism during antimicrobial therapy is a well-known and expected phenomenon. Almost all drugs that have been developed are associated with this problem. It has been shown that Gram negative bacteria, especially *Escherichia coli, Klebsiella* sp., *Serratia* sp., *Pseudomonas aeruginosa,* and *Enterobacter* and Gram positive cocci, particularly *staphylococci* and *Enterococcus* sp. are significantly more resistant to commonly used antimicrobials in developing countries, including Saudi Arabia (Farrar WE, 1985; Finland M, 1979; Murray et al., 1985; Olarte and Galindo, 1973; Qadri et al., 1987; Qadri et al., 1994; Shibl AM, 1992).

This decreased susceptibility may be due to the mechanism of mutation within a strain, or resistance transfer between organisms or species. Outbreaks of disease

due to multiresistant organisms have been reported to occur at a higher frequency in developing countries (Farrar WE, 1985; Murray et al., 1985). Resistance to antimicrobial agents is reported to be most common in countries where easy availability of drugs, without prescription, promotes heavy and indiscriminate use. Another factor that may lend a higher degree of resistance is uncontrolled use of antibiotics in animal feed.

Susceptibility data for *Escherichia coli* from Chile has shown that 44% patients were resistant to TMP-SX and similarly in Bangkok, 40% of isolates were resistant (Murray et al., 1985). Wilson and Guiney (1982) reported sepsis with TMP-SX resistant strain of *Escherichia coli* in a patient with cancer after prophylaxis with TMP-SX. TMP-SX resistance has also been reported in *Shigella* and *Salmonella* (Mathewson and Murray, 1983; Tiemens et al., 1984). At this hospital resistance to TMP-SX ranged from 45-53% over six years. These findings are in contrast to the U.S. where TMP-SX resistance is only found in 4-6% of *Escherichia coli* (Murray et al., 1985).

In a multicentre survey in seven Middle Eastern countries including Saudi Arabia, an investigation of community acquired infections found high rates of resistance among Gram negative organisms; for penicillin 86%, ampicillin 67% and amoxycillin 58%. Resistance to tetracycline and co-trimoxazole was seen to a lesser extent (Shibl, 1992). Higher rates of resistance were found at this hospital. For example, ampicillin resistance was found to be up to 72% in *Escherichia coli*, 100%

in *Klebsiella* spp. and *Morganella*, 42% in Proteus, 29% in *Salmonella* and 56% in *Shigella*.

Studies in developed countries have shown that antimicrobial resistance is of much smaller magnitude. A study in Sweden (Walder et al., 1994) tested the susceptibility of 880 blood culture isolates to 24 antibiotics. There was found to be no major development of resistance. Imipenem and the combination of pipercillin-tazobaclam were active against 95% of strains. 98% of strains were inhibited by ciprofloxacin and ofloxacin.

Several studies have been conducted at this centre in recent years to compare newer drugs for antibacterial activity. In one study, we compared the activity of six fluoroquinolones against 1000 clinical isolates. All six fluoroquinolones were found to have excellent *in-vitro* activity, inhibiting greater than 90% of *Escherichia coli* at an MIC of less than 0.03-0.5 mg/L, *K. pneumoniae* at 0.12-2.0 mg/L, *S. marcescens* at 0.12-2.0 mg/L and *P. aeruginosa* at 0.5-2.0 mg/L (Akhter et al., 1992). In another study the activity of the aminothiazolyl cephalosporin RU *K. pneumoniae, K. oxytoca, Enterobacter, P. mirabilis, Salmonella, Shigella* and *Morganella* at an MIC of 0.5-1.0 mg/L. All strains of *P. aeruginosa* and *Stenotrophomonas maltophilia* were resistant to RU 29,246 and 56% of enterococcal isolates were inhibited by 1.0-16 mg/L (Qadri et al., 1993). A recent study using the dual acting cephalosporin Ro 23-9424, was tested against 621 members of the *Enterobacteriaceae*, every single isolate was inhibited at an MIC ranging between

less than 0.003 -8 μg/ml. Its *in vitro* activity against coagulase negative *Staphylococcus* and *Enterococci* was also superior to that of commonly used drugs (Qadri et al., 1993). The effectiveness of these fluoroquinolones needs to be tempered with caution since resistance to these agents has also started to emerge.

In-vitro susceptibility testing of antimicrobial agents and monitoring the trends towards resistance is necessary in instituting or modifying the administration of effective therapeutic agents in the treatment of infections caused by antibiotic susceptible organisms. These tests are indicated for those organisms contributing to the infectious process whose susceptibility cannot be predicted from knowledge of their identity. This applies in particular, to Gram-negative fermentative and non-fermentative organisms, to some anaerobes, and to unusual and opportunistic species playing a pathogenic role. Antibiograms may also be determined for epidemiological reasons because the occurrence of an unusual antibiogram for a given species often assists in the recognition of common-source outbreaks and patterns of cross-infection. Another indication for performing these tests is the presence of bacteria that tend to develop resistance. This is especially true of Gram negative enteric bacilli which are commonly associated in serious and life threatening infections. Often, antimicrobial coverage or empirical therapy is initiated while laboratory studies are in progress. In such instances, knowledge of the general pattern of commonly isolated organisms in an institution is desirable which prompted analysis of data of the last 6 years. We found a significant increase in antimicrobial resistance in all members of the *Enterobacteriaceae.*

In addition to the use of antimicrobials in clinical practice other factors that might have contributed to the increase in resistance is that until recently antimicrobials were readily available without prescription in Saudi Arabia, this may have led to misuse or overuse. Since KFSH & RC is a referral tertiary care facility, patients seen here were treated previously, resulting in a variety of resistant bacteria. Another factor for increased resistance may be the large number of expatriates, from approximately 50 countries, that are employed in Saudi Arabia. Some of the resistant strains could possibly have been introduced into Saudi Arabia as normal flora by foreign workers or brought back from vacations in developing countries where resistant bacteria are found. Also till recently Saudis travelled to third world countries especially India for organ transplants such as heart and kidney due to the shortage of organs in this country. They often returned with chronic rejection of the organ and severe infections.

Surveillance of bacterial resistance trends are essential for a viable nationwide programme for shaping appropriate treatments and planning new strategies. This will aid in preventing infection and to administer empirical therapy before the offending organism is isolated and its antimicrobial susceptibility determined.

2.3. *Clostridium difficile* antibiotic associated colitis.

Introduction.

C. difficile antibiotic-associated colitis is of particular importance in tertiary care facilities where it causes severe infections in patients due to heavy drug regimens. *C. difficile* is the only pathogen commonly identified with antibiotic-associated diarrhoea or colitis (Bartlett, 1994). Hence, the epidemiology of this organism was also investigated and some methods for rapid detection.

The medical charts of 224 patients were examined in which *C. difficile* toxin had been isolated from culture. It was found that 158 (70%) of these patients were organ transplant, bone marrow transplant recipients or neutropenic patients who were on immunosuppressive therapies. Seventeen patients had renal disease, 14 suffered from heart abnormalities, 9 lung disease, 9 had liver disease, 6 suffered from cancer, 5 had bowel surgery and 15 suffered from other diseases. The antimicrobial agents administered to these patients included: metronidazole, vancomycin, sulphamethoxazole, ticarcillin, amikacin, ampicillin, cefazolin, imipenem, ceftazidime, trimethoprim, amphotericin B, gentamicin and fluconazole.

2.4. Evaluation of faecal leukocytes and pH in stools submitted for *C. difficile* toxin.

Introduction.

C. difficile is the main cause of antibiotic-associated diarrhoea and cytotoxic assays (CTA) are the standard method for detection. The literature shows that 33-50% of patients with *C. difficile* have faecal leukocytes. Therefore, to determine their diagnostic value, faecal leukocytes and pH were examined to determine whether they could be used as a rapid and presumptive test.

Method.

150 stool specimens submitted for *C. difficile* toxin were also examined for gross appearance, pH and faecal leukocytes using methylene blue stain. Briefly, a small amount of stool specimen was transferred to a clean slide and mixed with a drop of methylene blue stain.After 2-3 minutes the slide was examined at x100 for leukocytes and confirmed at x 400. Two or more leukocytes in two separate high fields were considered positive.

Results.

Of the 150 stool specimens tested 17 were found to be positive for *C. difficile* toxin by CTA. Faecal leukocytes were found in two stools that were positive for *C. difficile* toxin and also in two patients that were negative for *C. difficile* toxin by CTA. The pH was found to range from 6.0-8.6. Of the 17 stools, 14 were watery, 2 contained mucus and one had semi-hard consistency. There were 15

females and 5 males. The age range varied from 2 months to 70 years (Table 2.4.1.).

Table 2.4.1. Examination of faecal leukocytes and pH in patient stools.

No.	Positive *C. difficile* toxin	Leukocytes	pH	Age	Sex	Stool consistency
1	+	-	7.5	25	F	soft
2	+	-	7.5	17	M	soft
3	+	-	8.5	40	F	watery
4	+	-	7.5	21	M	watery
5	+	+	7.5	48	F	watery
6	-	+	8.0	36	M	watery/ mucus
8	+	-	7.5	48	F	watery
9	-	+	8.5	2	F	soft
10	+	-	8.5	41	F	soft
11	+	-	7.5	19	F	mucus
12	+	-	6.0	70	F	watery
13	+	-	6.0	17	F	soft
14	+	-	6.0	8 months	F	mucus
15	+	+	6.0	51	M	loose
16	+	-	6.0	17	F	semi-hard
17	+	-	7.5	34	F	watery/ mucus

Discussion.

Several authors have stated that faecal leukocytes are present in 33-50% of stool specimens from patients with *C. difficile* colitis (Bartlett, 1990; Fekety, 1990; Silva 1989). However, there was no correlation between faecal leukocytes and

positive *C. difficile toxin* in this study. There was also no relationship between pH and faecal leukocytes or *C. difficile* toxin. This is in agreement with a similar study by Marx et al. (1993). However, faecal leukocytes have been found to be present with many faecal pathogens and may increase the probability of recovering an enteric pathogen.

2.5. Can intestinal pathogens cause false positives with the latex agglutination test for *C. difficile* toxin?.

Introduction.

It is well known that enterotoxin producing strains of *C. difficile* toxin cause antibiotic-associated colitis. It is probably the most common cause of nosocomial diarrhoea which is nearly always associated with the use of antibiotics or other chemotherapeutic agents (George et al., 1982;Gilligan, 1986; Lyerly et al., 1988; Yannelli et al., 1988). It has been estimated that *C. difficile* may cause up to 25% of the reported cases of antibiotic associated diarrhoea and that the number of cases of colitis by this bacterium is second only to that of *Campylobacter jejuni*, which is the most frequent cause of bacterial diarrhoea in the Western hemisphere (Gilligan et al., 1981; Brettle and Wallace, 1984). The definitive laboratory diagnosis is based upon the culture of enterotoxigenic *C. difficile* and/or the detection of toxin A and B. The isolation and identification of the bacterium and assays for toxin production or detection of toxin in faecal samples are time

consuming and technically demanding (Chang et al., 1979; Bartlett, 1981). Since a majority of diagnostic laboratories lack the facilities to cultivate *C. difficile* or undertake cell cultures necessary for CTA, other methods like counter-immunoelectrophoresis, EIA and latex tests have been developed. The latter has generated much interest because of the ease of operation. In a previous evaluation of a commercially available LA, a high frequency of false positives was found (Qadri et al., 1989). Also, 16 of the 31 patients yielding false positives had a microbial pathogen/parasite. Thus the LA test was further evaluated for cross reactivity with stool specimens containing such organisms especially since in the developing world a wider range of such organisms are present.

Method.

Faecal samples from 107 patients that were found to contain an intestinal pathogen/parasite were selected for this study. For the detection of potentially pathogenic bacteria, the samples were inoculated on to enriched sheep blood agar (Brucella agar base with haemin and menadione), kanamycin-vancomycin-laked blood agar, cefoxitin-cycloserine agar, and thioglycollate broth for anaerobic cultures and MacConkey agar, Hektoen, sheep blood agar, and selenite broth for aerobic/facultative bacteria. All media were purchased commercially (Saudi Prepared Media, Riyadh, S.A.). The cultures were incubated at 35°C. A Forma Scientific glove box was used for anaerobes and regular incubators for facultative bacteria. Bacterial isolates were identified by standard techniques (Balows et al., 1991). Rotavirus was detected by EM and EIA. Direct microscopic examination

of stools as well as examination after concentration by formalin-ethyl acetate method was performed for the presence of intestinal parasites.

The commercial LA kit was purchased from Marion Laboratories Inc. (Kansas City, MO) and the test performed according to manufacturer's directions. For cytotoxic assay stool filtrates were prepared by diluting the specimen 1:5 with tryptose phosphate broth with 0.5% gelatin, gentamicin, and amphotericin B (Flow Labs, Irvine, Scotland), and centrifuging at 2500 rpm for 10 mins at 4°C. The supernatant was collected and 0.1 ml was inoculated into two tubes of diploid fibroblast (human foreskin) cell cultures. A third tube was used for neutralization and received 0.1 ml of supernatant and 0.1 ml of *C. sordelli* antitoxin (Biologics, FDA, Bethesda, MD). The cell cultures were incubated at 37°C and examined at 24 and 48 hours. The supernatant from a toxigenic strain of *C. difficile* was used as a positive control.

Results.

Faecal specimens from 107 patients which contained microbial pathogens were tested for potential cross-reaction with LA test designed for the detection of *C. difficile* toxin A. Thirty four of the specimens contained bacterial pathogens, 4 rotavirus and 69 had protozoan and metazoan parasites (Table 2.5.1.). Of these the supernatant from one stool specimen, containing *Salmonella enteritidis*, yielded a false-positive reaction with the LA test.

Table 2.5.1. List of microbial pathogens/parasites in stool specimens tested for cross reactivity with the LA test.

Organism	No. tested	LA reactivity
Campylobacter sp.	9	Negative
Salmonella sp.	16	1 false Positive
Shigella sp.	8	Negative
Aeromonas hydrophila	1	Negative
Rotavirus	4	Negative
Endolimax nana	12	Negative
Blastocystis hominis	13	Negative
Entamoeba coli	13	Negative
Giardia lamblia	9	Negative
Entamoeba histolytica	4	Negative
Entamoeba hartmanni	4	Negative
Trichomonas hominis	2	Negative
Chilomastix mesnili	3	Negative
Iodamoeba butschlii	2	Negative
Trichuris trichiura	2	Negative
Mixed (*Entamoeba coli, Endolimax nana B. hominis. T. trichiura*)	5	Negative

Discussion.

Although the LA test has been designed to detect the toxin A of *C. difficile*, the method has been evaluated by several investigators who questioned its specificity for this enterotoxin. Lyerly and Wilkins (1986) demonstrated that instead of toxin A the LA test detected an antigen that was produced by both toxigenic as well as non-toxigenic strains of *C. difficile*. Subsequently it was found that the latex also cross-reacted with *C. sporogenes,* proteolytic strains of *C. botulinum*, *Peptostreptococcus anaerobius* and *Bacteroides asaccharolyticus* (Brettle and Wallace, 1984; Kamiya et al., 1986; Borriello et al., 1987; Miles et al., 1988). Miles et al. (1988) tested the cross reactivity of broth culture filtrates of 739 anaerobic, facultative and aerobic bacteria, belonging to 78 species. They did not find false-positive reactions with any bacteria except those mentioned above. Since the organisms they tested did not include any enteric pathogens/parasites and since during an earlier study we had found that 16 of the 31 specimens yielding false-positives had such microbes, cross-reactivity was investigated in these organisms. However, only one of the sixteen specimens which had grown *S. enteritidis* gave a positive reaction by the LA test. This was deemed to be false-positive because further tests showed a negative result by the cytotoxic assay and negative culture for *C. difficile*.

Miles et al. (1988) showed that that in spite of the non-specificity of the LA test for toxin A of *C. difficile* and cross reactivity with 4 or 5 other anaerobes, the test has its merits. Several studies have found that the LA test was superior in

sensitivity to cytotoxic assay or culture for enterotoxigenic *C. difficile* but inferior in its specificity (Peterson et al., 1986; Kelly et al., 1987; Sherman et al., 1988) and that the accuracy of detecting *C. difficile*-associated diarrhoea could be improved by using the LA test in combination with cytotoxin assay or culture (Kelly et al., 1987). These observations suggest that the LA test could be used as a rapid screening test in developing areas with limited resources and for epidemiology studies. However, all positive findings should be correlated with the clinical picture of the patient and if possible confirmed by tests that specifically detect enterotoxin of *C. difficile*.

2.6. *Cryptosporidium parvum*

Introduction.

The first cases of human cryptosporidiosis were reported in1976. Characteristically, the diarrhoea is profuse and watery, it may contain mucus, but mainly blood and leukocytes. Fayer and Ungar (Fayer and Unger, 1986) reported that diarrhoea was the most common listed clinical feature (92%) followed by nausea and vomiting (51%), abdominal pain (45%) and low grade fever (36%).

Prevalence rates reported in surveys from Europe (1-2%) and North America (0.6-4.3%) are lower than those reported in surveys from Asia, Australia, Africa and Central and South America (3-20%). Recent studies summarized by Crawford and Vermund (Crawford and Vermund ,1988) suggest that the infection rate in individuals with diarrhoeal illness was 2.2% for individuals in industrialized countries and 8.5% for individuals in developing countries.

The first three cases of *Cryptosporidium parvum* in Saudi Arabia were reported at this institute in 1989 (Qadri 1989). These were found in three cases of diarrhoea in immunocompetent patients in which the sole pathogen was *Cryptosporidium parvum*. As a follow up to this report, specimens between July 1991 to July 1994 were examined for this pathogen.

Method.

583 stool specimens were examined for *Cryptosporidium parvum* between July 1991 to July 994. Specimens were examined for stool consistency, colour, presence of mucus, frank blood, or adult forms of intestinal parasites. Specimens were concentrated by the ethyl-acetate-formalin method and the presence of *Cryptosporidium parvum* confirmed by using modified hot acid-fast stain.

Result.

From a total of 583 patient stools examined for *Cryptosporidium parvum* only 4 patients were found to be postive. These were in 4 male patients aged 6, 8, 13 and 22; two of the patients were immunocompromised.

Discussion.

Disease due to *Cryptosporidium parvum* appears to occcur worldwide as a zoonosis, with transmission via the faecal-oral spread of oocysts that are infective when passed. Although previously reported in immunocompromised patients only, almost one third of the reported cases have been described in immunologically intact patients, occurring mostly in veterinarians and animal handlers.

The incidence of *Cryptosporidium parvum* appears to be very low compared to other countries which could be due to a number of reasons. Due to inexperience in detecting *Cryptosporidium parvum* and the lack of controls there may be underestimation of this organism The pathogen may also be misdiagnosed for another disease or improvements in methodology are needed for

improved detection. Another possibility is that the organism may be minimal in the community since there is very little contact with animals and very few people keep pets which is considered undesirable.

CHAPTER 3. Epidemiology of Enteric Viruses in Saudi Arabia

The epidemiology of gastrointestinal adenovirus infections has not previously been examined in the Saudi population. Therefore, adenoviruses were detected using three methods and typed in order to determine their prevalence and the adenovirus types present in this community. Their significance and their characteristics are presented in this chapter.

3.1. Is there a need for the detection of adenoviruses in the stools of the Saudi population?

Introduction.

Rotavirus is most commonly associated with gastroenteritis and screened for by most diagnostic laboratories. However, adenovirus type 40 and 41 have also been strongly linked with gastroenteritis (Uhnoo et al., 1990). Diagnosis is usually by EM, as serotypes 40/41 are difficult to grow in culture. Thus efforts have been made to develop simpler and more rapid tests such as latex agglutination (Sanekata et al., 1990). Patient stools routinely submitted for Rotavirus detection by clinicians were simultaneously tested for adenovirus.

Method.

Faecal material from 139 patients were tested for the presence of rotavirus and adenovirus. Twelve specimens from normal, healthy persons were also collected and used as controls.

The Abbott rotazyme II kit (Abbott, North Chicago, Illinois) was used to test for rotavirus. This is an enzyme linked immunoassay for detection of the antigens. The test employs polystyrene beads that are coated with guinea-pig anti-rotavirus antibodies. Briefly, anti-rotavirus monoclonal antibodies were adsorbed to a solid phase. Next a 10% stool suspension was added for 60 min incubation at 37° C; if virus was present, it became bound to the rotavirus antibody. A second antibody conjugated to an enzyme was added next after washing to give a colour reaction with O-phenylene diamine that was read spectrophotometrically.

Adenovirus antigen in faeces was tested by a latex agglutination method (Adenolex, Orion Diagnostica, Espoo, Finland). This contained latex reagent (particles coated with adenovirus antibodies) and latex control reagent (particles coated with immunoglobulins). Briefly, one drop of each reagent and control reagent was deposited on separate black areas on wallboard slides. An equal amount of 10% faecal suspension was added to each of the two spots and mixed with the reagents. The slide was rocked for two minutes and results recorded. Both methods were performed as per manufacturer's instructions. Acute diarrhoeal patients were tested in patients from 3 months to 41 years.

Results

Of the 139 acute diarrhoeal specimens examined from 139 patients for rotavirus and adenovirus, 20 (14%) were positive for rotavirus and 11 specimens (8%) were positive for adenovirus. Analysis of the 139 patients by age group showed that the majority of stol samples were received from under 6 years of age (84 patients)(Table 3.1.1.). Two patients were positive for both rotavirus and adenovirus. Three specimens tested for adenovirus showed non-specific reactions and could not be interpreted.

Medical records of all patients with positive viral cultures were reviewed. All the patients had gastrointestinal symptoms and none were positive for bacterial pathogens or ova/parasites. Seventeen of the 20 patients positive for rotavirus were under 6 years of age. However, patients positive for adenovirus ranged in age from 10 months to 41 years.

Most of the positive adenovirus cases were from young children or immunocompromised patients. There was an even distribution of positive cases between male and female patients. None of the 12 control specimens were positive for adenovirus 40/41.

Table 3.1.1. Characteristics of patients with adenovirus and rotavirus gastroenteritis at KFSH & RC.

Characteristics	Adenovirus	Rotavirus
No of patients	139	139
No. of patients positive	11 (8%)	20 (14%)
Male/female ratio	6:5	9:11
Age (years)		
less than 6	5	17
7-18	0	0
19-55	6	3
Inpatients:outpatients	5:6	5:15

Age group of 139 patients examined for rotavirus and adenovirus.

Age group (yrs)	**Number**
less than 6	84
7 - 18	34
19 - 55	11
over 55	10

Discussion.

The prevalence of adenovirus can be up to 50% of the rotavirus frequency. This was found to be the case in this study, in which 14% of specimens were positive for rotavirus and 8% positive for adenovirus type 40/41.

Rotavirus has seasonal variations in countries witha temperate climate but

adenovirus appears to remain the same throughout the year (Haukenes et al., 1989). Also, since both infections follow a similar clinical course initially, it is possible that adenovirus infections are overlooked. Several reports have appeared on the incidence and significance of rotavirus infections in Saudi Arabia (Walker et al., 1984; Arya et al., 1986; Mohammed et al., 1994). However, adenovirus-associated gastroenteritis has not been reported in this country. Yolken and Francillin (Yolken and Francillin 1985) have reported that patients with necrotizing enterocolitis and infected with adenovirus have an increased morbidity and prolonged hospitalization. Other adenovirus serotypes have also been implicated in viral gastroenteritis.

Rotavirus infections were found to occur primarily in children under 6 years of age, whereas a much wider fluctuation occurred with enteric adenovirus infections, ranging from 10 months to 41 years. It would appear that screening stools for adenovirus in patients with suspected viral aetiology is warranted. But further studies are needed to determine the true incidence and epidemiology of adenoviruses in the Saudi population.

3.2. The clinical characteristics of patients with adenovirus infections at a Tertiary Care Hospital in Saudi Arabia.

Introduction.

Opportunistic infections in children and adults may be caused by bacteria, fungi, and viruses. Such infections in the immunocompromised host are particularly frequent and often fatal due to an impaired immune system. Adenoviruses are among the many agents that take advantage of an impaired immune system to set up persistent and generalized infections in the immunocompromised host. These infections are difficult to treat, tend to be long term and can cause death. Adenoviruses are endemic in paediatric populations, but outbreaks can occur with certain body systems and in selected populations.

Prior to undertaking a prospective large scale study of adenoviruses in diarrhoeal patients. Although adenoviruses other than types 40/41 are not normally associated with diarrhoea, they have been recovered from virtually every organ system and have been associated with many clinical syndromes. Outbreaks can also occur in association with certain body systems and in selected populations. The medical records of 83 patients were examined in which adenovirus had been isolated by culture. The characteristics and clinical data are presented.

Results.

The medical records of 83 patients were examined in which adenovirus had been isolated in the virus laboratory. The majority of patients (46) were under 5 years of

age and 24 were under the age of nineteen. The proportion of males to females was 49 to 34 respectively. Most of the patients presented with respiratory infections (53) but 19 patients exhibited symptoms of both respiratory infections and diarrhoea. Fourteen of the 83 patients died in which 6 patients had leukaemia, 3 were immunocompromised, 3 had Hodgkins disease, 1 suffered from heart disease and 1 was a transplant patient.

Table 3.2.1. Characteristics of patients with adenovirus infection.

Characteristic	Number of patients
Total no. of patients	83
no. of males	49
no. of females	34
Age (years)	
0 - 5	46
6 - 19	24
20 - 35	8
36 - 55	4
greater than 55	1
Adenovirus infection:	
Respiratory	53
diarrhoea and vomiting	10
respiratory and diarrhoea	19
haemorrhagic cystitis	1
Virus isolated from :	
stool	33
respiratory	40
urine	4
eye	6

Table 3.2.2. Clinical history of patients with adenovirus infections

Primary disease	Number	Outcome	
		Recovered	Died
leukaemia	13	7	6
immunocompromised	10	7	3
heart disease	4	3	1
transplant	8	7	1
Hodgkins disease	9	6	3
pneumonia	15	15	-
diarrhoea/vomiting	10	10	-
tosillitis	5	5	-
surgery	4	4	-
poliomyelitis	1	1	-
asthma	1	1	-
otitis media	2	2	-
haemorrhagic cystitis	1	1	-

Discussion.

Adenoviruses have been recovered from virtually every organ system of humans and have been associated with many clinical syndromes. The spectrum of symptoms also differs; in normal children, these viruses cause mild to moderately severe upper respiratory illness (URI) and some gastroenteritis. However, in children with adenovirus-related immunocompromised host disease (ICHD), lung, liver and kidney involvement is common and often has a fatality rate of 55%.

Adenoviruses can be excreted in stools for weeks or months after initial infection and can also become latent in the body. These features are compatible with the

apparent long term presence of adenoviruses in the intestinal and urinary tracts and with their possible contribution to disease in the immunocompromised host.

A comprehensive study of Australian men showed that 54% of diarrhoeal disease in AIDS patients were caused by viruses and that 37% of these were adenovirus related. Adenoviruses cause active infection in an estimated 12% of patients with clinical AIDS and 45% of these cases result in death within two months of the initial detection of adenovirus (Cunningham et al., 1988). At this hospital, the majority of patients were immunocompromised and 44 of the 83 patients were under 5 years of age. This is consistent with the fact that adenoviruses cause primarily childhood diseases.

The source of virus in these infections has caused much controversy. The presence of type-specific antibody in organ transplant patients before transplantation indicates that the virus was previously present in the body due to latency, thus the source of virus is likely to be endogenous. In the case of liver and renal transplant patients, it has been found that the virus is latent in the donor but reactivated in the recipient. Infections in the immunocompromised host have presented new and severe problems due to increase in organ and tissue transplantation, AIDS and premature babies (Koneru et al., 1990; Norris et al., 1989).

Due to these factors and that this is a tertiary care facility with a large number of immunocompromised patients. A large scale study was carried out to document the prevalence of adenoviruses in this community, determine the serotypes present and their relation to type of patients.

3.3. Epidemiology of Gastrointestinal Adenovirus Infections in Saudi Arabia.

Introduction.

Adenovirus particles are seen in approximately 5-17% of cases of gastroenteritis in children up to 5 years of age. Chanock (Chanock 1974) has estimated from serological studies that the average individual undergoes a minimum of two or three clinical episodes of adenoviral infection during childhood.

There have been no studies on the prevalence and morbidity of adenovirus gastroenteritis in Saudi Arabia. Therefore, a prospective study was carried out to determine the incidence and distribution of adenovirus serotypes in diarrhoeal specimens from the Saudi population.

Method.

Specimens.

3,000 diarrhoeal stool samples were collected prospectively from patients of all ages at King Faisal Specialist Hospital and Research Centre from June 1992 to October 1994. Specimens were collected daily from the Microbiology, Parasitology and Virology sections. All loose and watery diarrhoeal stools were collected and all patient details and patient history recorded. All results obtained on the stool tests were also obtained and recorded.

Specimens for electron microscopy (EM) were suspended in sterile distilled water. The suspension was filtered and the supernatant centrifuged at 25,000 rpm for 1 hour at 4°C in a refrigerated Beckmann L7-55 ultra-centrifuge. Formvar/carbon grids (200 mesh) were prepared using negative staining and examined on a Jeol electron microscope (20,000-30,000x).

Specimen processing.

Stool specimens for culture were centrifuged at 2500 rpm for 15 minutes and the supernatant inoculated into an A549 cell line for respiratory adenoviruses and HEK-293 cell line for enteric adenoviruses. Cell lines were maintained with L-15 maintenance media (containing 2% foetal bovine serum, 1% gentamicin (5,000 μg/ml), 1% amphotericin (100 μg/ml, and 1% 200 mM glutamine). Cultures were maintained for two weeks at 37°C and read on alternate days for adenovirus cytopathic effect. Cultures were fed with fresh media every 4 days. Positive cultures were first confirmed using immunofluorescent monoclonal antibodies (Dako). Confirmed positive adenoviruses were then typed using neutralizing antisera (rabbit).

Results.

Adenovirus detection.

The 3,000 diarrhoeal specimens were received from 2574 patients. A total of 220 adenoviruses were detected, a prevalence of 8.5%. There were 128

male patients and 92 female patients. All age groups were represented but most infections were detected in children 5 years or less (Fig. 3.3.2.). There was no significant seasonal variation noted with the presence of adenovirus infection. One stool specimen was received from 2258 patients, 2 specimens from 246 patients, 3 specimens from 46 patients, 4 specimens from 13 patients, 5 specimens from 7 patients, 6 from 3 patients and 7 specimens from 1 patient. 175 patients were found to have only adenovirus in their stools (table 3.3.1.).

Serotyping

Serotyping of adenovirus isolates showed that type 40/41 was encountered most frequently (57 specimens) followed by serotypes 1 (20 specimens), serotype 2 (22 specimens), serotype 5 (20 specimens), and serotype 3 (13 specimens). Other less frequent serotypes found were types 4,12,15,17,20,22,24,26,27,and 28. Thirty four specimens did not type since they were detected by non-immune EM only and did not grow in culture. Some of these may have been type 40/41 but possibly due to antigenic drift or recombination were not detected (Fig 3.3.3.). Van der Oort et al. (1989) identified a new genome type in which change correlated with an alteration in one of three neutralizing epitopes. This observation has implication for the appearance and spread of new genome types.

Controls.

83 stool specimens taken from healthy adults and children showed that only 2 patients had adenovirus in their stool; 1 of type 3 and 1 of type 15.

Clinical Data.

Examination of patient data showed that of the patients with adenovirus present in stools: 21 had leukaemia, 6 cancer, 32 were diagnosed with other infections, 8 had inborn errrors of metabolism, 4 CNS disorders, 14 had diseased organs such as heart, kidney and liver; 6 were transplant patients, 1 had severe combined immunodeficiency, 1 patient had HIV and 28 had other minor underlying diseases. 99 patients had no evidence of underlying disease.

Table 3.3.1. Analysis of Positive Adenovirus Specimens

Age group	No. of specimens	No. of positives
0 - 5 yrs	1154	127 (11%)
6 - 19 yrs	598	43 (7%)
20 - 55 yrs	950	38 (4%)
over 55 yrs	298	12 (4%)
Total	3000	220
Male : Female	**1682 :1318**	**128:92**

Patient type	**Number**
leukaemia	21
cancer	6
infections	32
inborn errors	8
CNS disorder	4
diseased organ	14
transplant	6
SCID	1
HIV	1
minor/vomiting	28
other	99
Total	220

Other pathogens present with adenovirus in stool specimens:

Pathogen	Number
adenovirus alone	175
adenovirus and bacteria	17
adenovirus and parasites	20
adenovirus and viruses	8
Total	220

Fig. 3.3.2. Distribution of Adenovirus by Age Group.

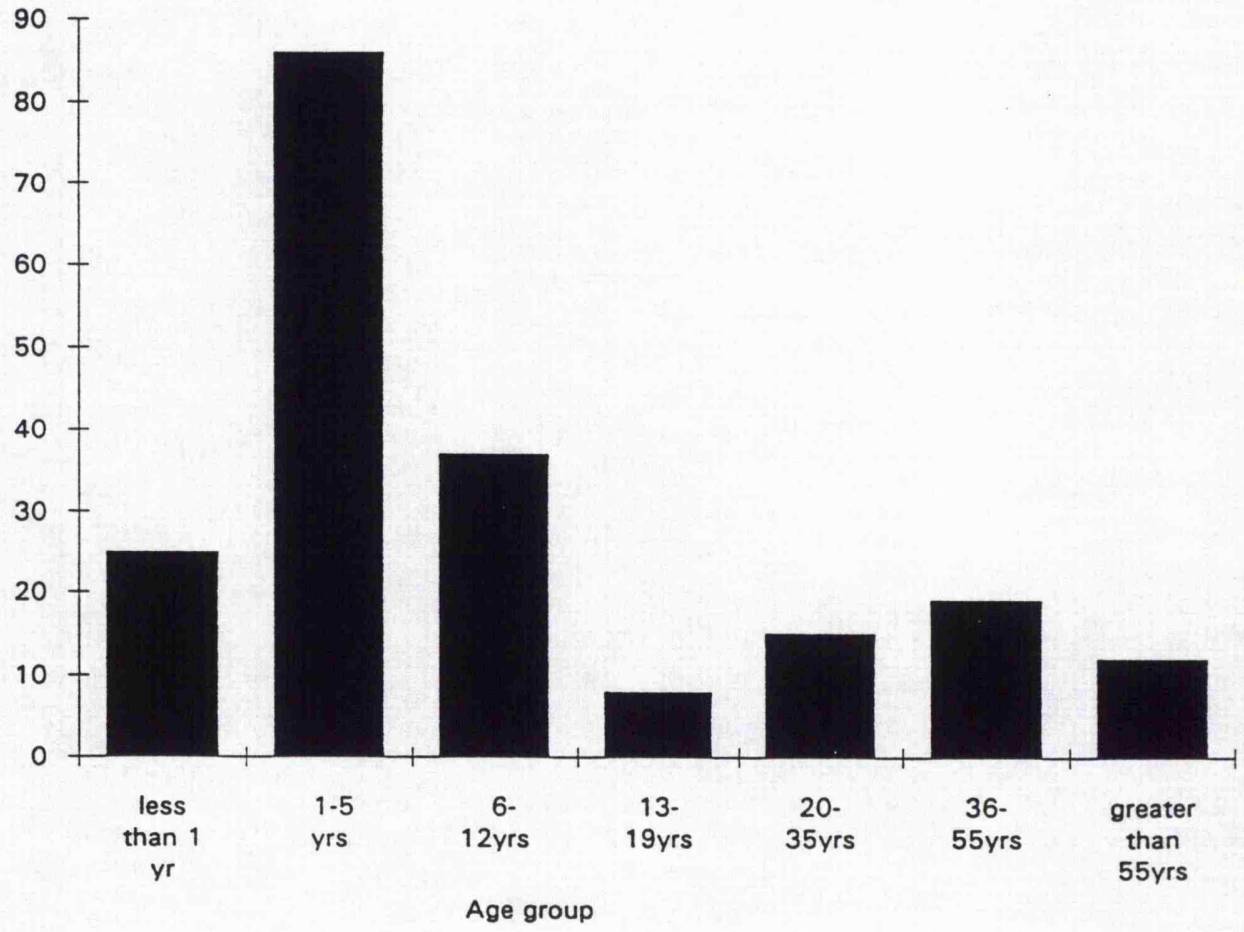
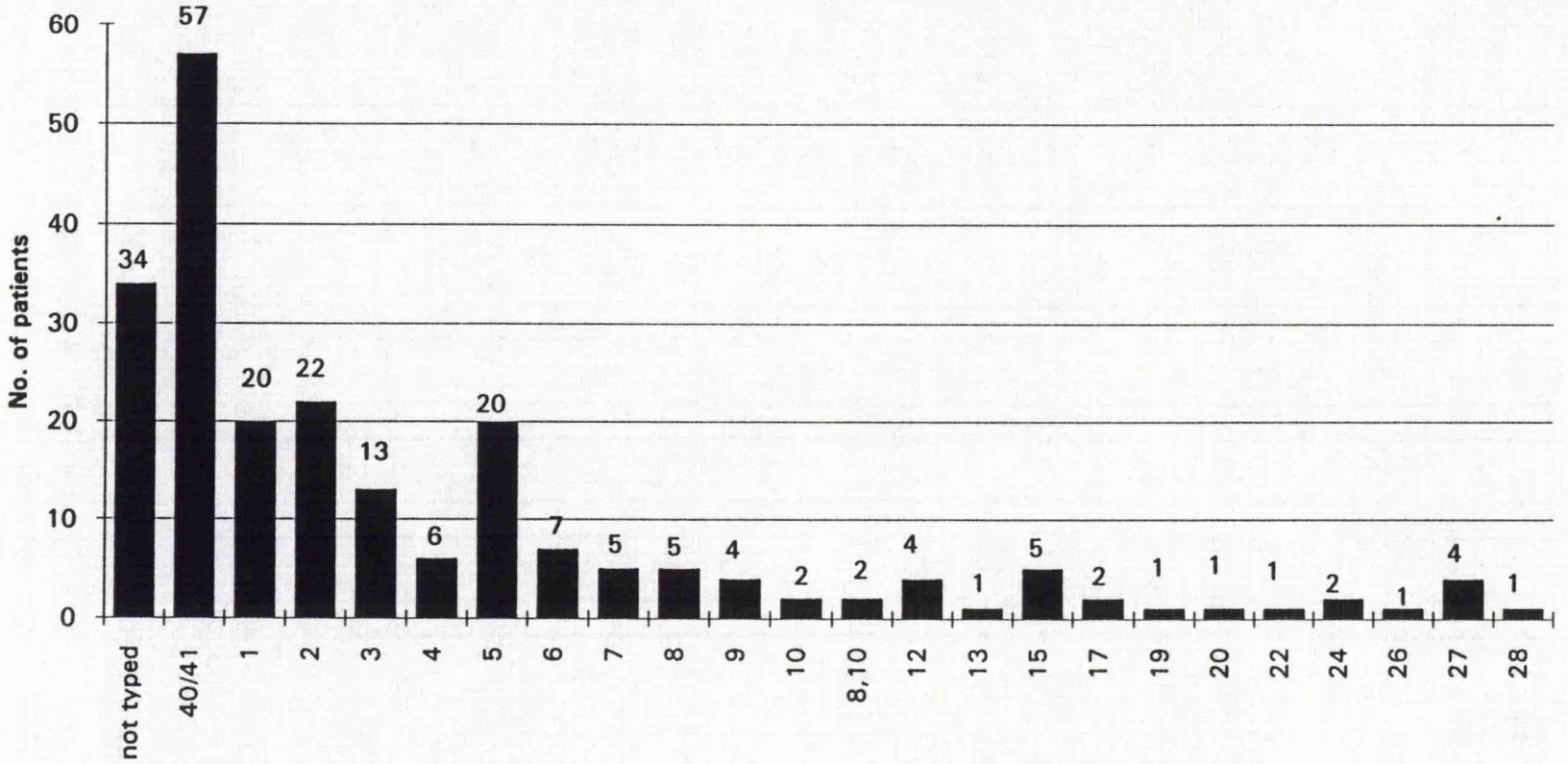


Fig. 3.3.3. Distribution of adenovirus serotypes in patients at KFSH & RC.



Discussion.

Acute infectious diarrhoea is a major health problem throughout the world and viral gastroenteritis has been found to be the second most common clinical entity in developed countries behind viral respiratory tract illness (Kapikian, 1976). In the U.S.A. diarrhoea may occur in each member of the population 1-3 times per year, while those living in developing countries may have 9 illnesses a year (Guerrant 1984).

There has been little documentation of the role of adenoviruses in disease states in the developing countries, including Saudi Arabia. The need for such information is more pressing in immunocompromised patients because of the biological properties of adenoviruses. They are pathogenic for diverse tissues, they can be spread easily and cause a wide variety of clinical syndromes (Hierholzer, 1992).

The relationship between adenoviruses and diarrhoea has a long and complicated history since adenoviruses replicate efficiently in the intestine and are excreted in the stool. In some studies adenoviruses have been detected in equal frequencies among patients with gastroenteritis and asymptomatic controls whereas in other studies significantly higher isolation rates have been found. Some serotypes, like adenovirus 12, 18, and 31, have a definite tropism for the alimentary tract and have been reported

to cause diarrhoea in children (Schmitz et al., 1983). Other adenoviruses like type 3 and 7, cause gastrointestinal symptoms but only as a part of a systemic infection with accompanying respiratory symptoms (Wadell, 1987). However, types 40 and 41 have been demonstrated to account for the majority of diarrhoea cases associated with adenoviruses.

In this study 175 diarrhoeal stools were found to have adenovirus as the only infectious agent present. Although diarrhoea can be the result of many non-infective diarrhoeas some of these cases may have involved adenovirus.

Adenovirus types 1,2,3,5 and 7 are the most common adenoviruses found in normal children. Children with SCID are prone to develop severe infections with the most frequently occurring persistent adenoviruses. In subgenus A types 12, 18, 31 have been found in SCID patients with pneumonia, hepatitis, diarrhoea and acute haemorrhagic cystitis. Bone marrow transplant recipients are children or young adults with aplastic anaemia, leukaemia, cancers or congenital immunodeficiencies. They are exposed to the more common serotypes, thus types 1, 2, 5, 17 and 31 predominate (Hierholzer, 1992).

Tertiary care facilities in particular need to screen for adenoviruses due to the large number of immunocompromised, organ transplant and BMT patients but adenoviruses have been largely ignored at medical facilities in

this country. Camitta et al. (Camitta 1994) have shown that 21% of BMT recipients have positive adenovirus cultures after BMT and that one-third of these had definite or probable adenovirus disease. Another study (Troussard 1993) showed that adenoviruses are a cause of enteritis in patients undergoing BMT and are underdiagnosed and can be confused with GVHD. This institute has a liver transplant programme and a study at Pittsburgh (Michael 1992) has shown that of 484 liver transplant recipients, 49 had 53 episodes of adenoviral infection . These were recovered from the gastrointestinal tract, liver and lung. Serotypes 1,2 and 5 were most often recovered and invasive adenoviral infection occurred in 20 children leading to death in 9 of them.

In this study serotypes 40/41 (30%) were the most prevalent followed by 1, 2, 3 and 5. Three patients with type 40/41 had a dual infection with adenovirus type 1,2 and 15. Two patient isolates cross reacted with type 8 and 10 antisera indicating an antigenic relationship. A Canadian study (Brown, 1990) in which analysis of adenovirus serotypes associated with gastroenteritis showed that the predominant types were type 31, 40 and 41 which accounted for 70% of isolates. The remaining isolates were typed as 1,2,3,5,7, and 12. A similar study in Australia (Mickan 1994) in which 226 adenoviruses from diarrhoeal patients were typed and found that type 40 and 41 accounted for 20% and 40% respectively and that types 1,2,3,5,6,7, and 31 comprised the remainder. However, in our study type 31 was not

detected.

Statistical analysis of the data showed that of 397 specimens submitted for bacterial culture, 26 were also found to be positive for adenovirus. In 599 specimens that had been submitted for bacterial culture and ova/parasites only, it was found that 25 specimens had baceria only, 32 specimens had parasites only and 33 specimens had adenovirus only. Stool specimens requesting for rotavirus, bacteria and ova/parasites showed that 17 had rotavirus alone, 17 bacteria alone, 6 parasites alone and 21 had only adenovirus. Only two of 83 controls were found to have adenovirus in their stools indicating a low carriage rate in healthy persons. Adenovirus isolation is not requested by physicians at this institute in diarrhoeal specimens and these results indicate that those infections are being overlooked.

It is becoming increasingly evident that there is a need to study this group of viruses more intensely, especially since new serotypes have been implicated in viral gastroenteritis and overwhelming infections in immunocompromised patients. They can also become latent and be reactivated or excreted for long periods of time after infection. Patients with suspected viral aetiology should be routinely screened for adenovirus in order to determine the true epidemiology of adenovirus infections and the most prevalent serotypes in Saudi Arabia.

3.4. Serodiagnosis of Adenovirus Infections.

Introduction.

Serodiagnosis of adenovirus infections can be performed for all syndromes since they share group specific antigen epitopes. These can be detected by complement-fixation (CF) or enzyme-immunoassay (Zuckerman et al., 1987; Hierholzer, 1991). Acute and convalescent sera are submitted 2-4 weeks apart. Serology can also be of epidemiological importance or of clinical interest in establishing an association between an unusual severe illness and adenovirus infection.

Method.

Serum samples from 565 patients were analysed for the presence of adenovirus antibodies during 1987-1992. All sera were tested by complement-fixation test (CF) using a standard microplate method. A 1:4 dilution of serum in barbital buffered diluent (Flow Labs, High Wycombe, UK) was prepared and heat inactivated at 56°C for 30 mins. Serial dilutions in 'U' well microplates (Dynatech, Chantilly, VA, USA), starting at 1:8 to 1:2048 were made, and a second dilution of 1:8 to 1:32 was prepared on the same plate to test for anticomplementary activity (control cells). Diluted antigen (Whittaker MA Bioproducts, Walkersville, NJ, USA) was added to test wells and BBD was added to control wells to maintain equal volumes. Guinea-pig complement (Whittaker MA Bioproducts) diluted in BBD was added to all wells, the plates were mixed and incubated overnight at 4°

C. The following day washed sheep red blood cells, sensitized with haemolysin (Whittaker MA Bioproducts), were added to each well, mixed and incubated at 37°C for 45 mins. with mixing after 15 and 30 mins. Plates were centrifuged for 3 min. at 300 x g and read with the endpoint at 25% haemolysis. Optimal antigen, complement and haemolysin dilutions were determined by chessboard titrations. Sera not immediately tested were stored at -20°C until such testing could be performed. Acute and convalescent specimens were tested on the same day.

Results.

Single and paired sera from 565 patients were examined during January 1987 to January 1992. A titre of less than 1:8 was considered negative since 98% of our patient population had titres less than this, a titre of 1:8 or 1:16 as exposure to the virus and greater than 1:16 as indicating recent or current infection (Table 3.3.1.).

Table 3.4.1. Prevalence of adenovirus antibody titres in a 5-year period at a tertiary care center.

No. of patients	Titre
445	less than 8
75	8
32	16
2	32
5	64
2	128
2	256
2	512

A titre of greater than 16 or a four-fold rise was considered indication of adenovirus infection.

There were 13 patients positive for adenovirus antibodies with titres greater than 1:16 (Table 3.4.1.). Of these, 11 patients were immunocompromised; 5 of them died. There was one primary diagnosis of adenovirus pneumonia and another patient with adenovirus heamorrhagic cystitis. Seven patients had respiratory distress with fever and two patients presented with persistent diarrhoea.

Eleven of the 13 patients had negative bacterial cultures, three patients were negative for fungal cultures and two patients were negative for mycobacteria. Viral serologies were carried out on eight patients of whom five were negative for influenza, parainfluenza, respiratory syncytial virus and mycoplasma. A further three patients were negative for herpes simplex, cytomegalovirus, and Epstein-Barr virus. Three of the patients failed to respond to multiple antibiotic therapy and two patients failed to respond to acyclovir.

Only four cases had follow-up serology; where one patient had a four fold rise from1:64 to 1:256; one patient had a rise from 1:128 to 1:256 and two patients had a stationary titres of 1:128 and 1:32. Although the mere demonstration of a high antibody titre in the remaining patients is of limited diagnostic value, the data was evaluated in the light of the clinical findings. Twelve of the patients were under 12 years old and 10 of the 13 patients were male. There was only one adult with serological evidence of adenovirus who had been admitted for a renal transplant.

Table 3.4.2. Clinical data of 13 patients with positive adenovirus antibody titres.

No.	Age/Sex	Primary diagnosis	adenovirus associated Acute signs and symptoms	Convalescent titre	titre	Outcome
1	3M	vitamin D sensitive Ricketts, restrictive lung disease.	acute respiratory distress, Fever.	32	N/A	Died
2	3F	Complex cyanotic heart disease	endocarditis	128	128	Died
3	4M	Wilms tumour stage III	respiratory distress persistent diarrhoea	64	N/A	Recovered
4	4M	Acute lymphoblastic leukaemia	respiratory distress	128	N/A	Died
5	5M	T-cell deficiency	Fever. abdominal pain	512	N/A	Recovered
6	7F	Adenoviral pneumonia	respiratory distress, cough	512	N/A	Recovered
7	7M	Lymphoblastic lymphoma of abdomen	distal metastasis to CNS and bone marrow	64	512	Died
8	7M	combined immuno-deficiency	Fever, unresolving	64	N/A	Died
9	8F	Immune deficiency	respiratory distress, fever	32	32	Recovered
10	8M	Hodgkins disease	spiking temp., chills	64	N/A	Recovered
11	2M	Thymic mass enlargement	Fever, respiratory distress	64	N/A	Recovered
12	7M	Neck mass, viral lymphadenitis	Fever, diarrhoea	64	N/A	Recovered
13	28M	Renal transplant	Haemorrhagic cystitis-viral	128	256	Recovered

Discussion.

The diagnosis of causative agents such as adenovirus is often sought by serological methods because of the time, cost and technical expertise needed for cultivation of viruses. Although rapid tests are available for direct antigen detection in clinical specimens (EIA, LA, PCR), they are time consuming and beyond the scope of many small laboratories, especially when screening for multiple pathogens (Martin and Kudesia, 1990; Allard et al., 1990). The quality of the clinical specimen is also a major factor; some viruses cannot be cultivated and specimens must be collected during active viral shedding (Fenner and White, 1976). In contrast, screening of large numbers of sera, for antibodies to multiple organisms and for epidemiological purposes remains both time- and cost-effective. A previous report has shown that adenovirus accounted for 13% of viral respiratory disease between 1967 and 1976 (WHO report, 1980). A prevalence of 6% was previously reported in Saudi Arabia but the study examined only a very small patient population (Al-Hadramy et al., 1988). Another study of tonsillitis and respiratory infections of viral aetiology in Saudi Arabia showed adenovirus to be the most dominant respiratory pathogen in both adults and children (Hosain et al., 1988). In this patient population evidence of recent infection was found in only 2.3% but evidence of exposure in 21% of patients.

Shinozaki et al (1987) examined 322 serum samples for enteric adenoviruses in various age groups. They found the incidence to be 20% in children between 1

and 6 months old and 50% in those 37-48 months old; 48% of adults and 10% of the aged had antibodies using a neutralization test. Another study showed that viral pulmonary infections are a major cause of morbidity and mortality in solid organ transplant recipients. The herpes group of viruses caused most viral infections in such patients but adenoviruses, RSV and HIV remained major causes of pneumonitis (Anderson et al., 1990).

Although serological changes are important for definitive diagnosis, it must be emphasized that serological investigations have their limitations. For example, Landry et al. (1987) reported a bone marrow recipient with rapidly fatal gastroenteritis. Adenovirus infection was confirmed by electron microscopy but CFT was negative. The main disadvantage of the CFT is its low sensitivity; it can detect infection in only 50-70% of cases in general and less than 50% of cases in small children.

The low number of specimens sent for serodiagnosis causes underestimation of adenoviral infection as a serious illness as the low rate of convalescent specimens after an initial positive specimen shows. However, the absence of other infecting organisms in these cases indicates that greater emphasis should be given to this virus by clinicians since these figures may represent diagnoses of adenovirus infection that were overlooked. The low rate of positivity may be a result of patients not having viral serology performed, but instead having the diagnosis made on the basis of electron microscopy and viral culture. The diagnosis of infection is best performed by a combination of culture and serology (Hierholzer,

1991).

Our result are similar to the study of Zahradnik et al. (Zahradnik et al., 1980) who described 15 immunocompromised patients with evidence of adenovirus infection. All cases were associated with high fever, 11 cases had pulmonary complaints, and 9 patients died. It is concurred with them that adenovirus infection should be considered in the aetiology of severe overwhelming illness, especially in the immunocompromised host.

3.5. Comparison of biotinylated DNA probe with current methods for the detection of adenovirus in diarrhoeal disease.

Introduction.

Although the prevalence of rotavirus is well established in both developing and developed countries, there is relatively little data on the prevalence of adenovirus in gastrointestinal infections (Herrmann et al., 1988; Puerto et al., 1989; Akhter and Qadri, 1992). This has primarily been due to the lack of available tests that were available for adenovirus. However, rapid developments in technology have resulted in many complex virus isolation methods being replaced by new and rapid tests, making more convenient routine testing possible (Grandien et al., 1987; Wood et al., 1989). These newer methods include DNA probes, EIA, and latex agglutination.

Nucleic acid hybridization methods have been developed and used to identify microorganisms in foods. Tests performed on mixed cultures save the time required to establish pure cultures. Enterotoxigenic or invasive strains of foodborne bacterial pathogens are detected with probes that identify genes responsible for virulence. Hybridization tests signal the presence or absence of a particular strain or an entire genus and are especially well suited for screening foods for specific pathogens. However, hybridization tests require the presence of 10^5 to 10^6 cells to yield a positive result, thereby limiting sensitivity and necessitating a time-consuming growth step (Hill and Keasler, 1991).

The ability of a commercial biotinylated DNA kit for determining the presence of adenovirus in stools was compared with other methods currently available.

Method.

Acute diarrhoeal specimens from 245 patients were processed for the presence of adenovirus by culturing in appropriate cell lines. A direct suspension was used for latex agglutination, EIA, EM, and DNA probe. Stool specimens from 30 normal, healthy volunteers were collected and used as controls, including 8 specimens from children under 5 and 22 adults. All specimens were also tested with LA, EM, EIA, and DNA probe.

The human lung carcinoma (A549) cell line was grown in Hams F-12 medium with 10% fetal bovine serum, 100 U/ml penicillin, and 100μg/ml streptomycin. Clinical samples were prepared for cell culture and inoculated in cell culture tubes within 14 hours. Briefly, stool filtrates were prepared by diluting the stool specimen 1:5 with tryptose phosphate broth with 0.5% gelatin, gentamicin, and amphotericin B (Flow Labs, Irvine, Scotland), and centrifuging at 2500 rpm for 10 mins at 4° C. The supernatant was collected and 0.1 ml inoculated into cell cultures. Each specimen was inoculated in duplicate. The cell cultures were incubated at 37°C and read on alternate days for two weeks. Adenovirus cytopathic effects were confirmed by immunofluorescence using polyclonal antibodies (Novo Nordisk, Cambridge, UK).

Biotinylated probe kits (Pathogene) were purchased from Enzo Biochem, Inc. (New York, NY, USA) and used for adenovirus detection directly on stool specimens as well as positive cell cultures. The probe used was group specific and

hybridized wih adenovirus types 4, 5, 7, 11, 20, 40 and 41. The test was performed according to manufacturer's directions. Briefly, the specimens were fixed to a glass slide and a drop of bioprobe added. Specimens were covered with a coverslip, and placed on a heating block for 2-3 minutes at 92°C. The slide was removed and left to hybridize for 15 minutes. Four drops of probe solution were added for 7 mins. and then rinsed. The conjugate was added and allowed to react for 10-15 minutes at room temperature. After washing, 4 drops of chromogen substrate was added and left at RT for 15 min.. The slide was rinsed, mounted and examined under x100 and x400 magnifications. A positive was indicated by brick red deposits in cells.

Electron Microscopy. Specimens for EM were processed by filtering stool suspensions through 0.22μm pore size filter and centrifuged for one hour at 37,400 x g. Grids (200 mesh formvar/carbon coated) were prepared by negative staining and examined by a Jeol electron microscope at x20,000-x30,000.

EIA and LA. The EIA adenovirus kit (Dako, Cambridge, UK) was used to examine stool samples and CPE positive cell lines for the presence of viral antigen. The test was carried according to manufacturers instructions. Adenovirus was also detected by an LA method (Adenolex, Orion Diagnostica, Espoo, Finland).

For positive controls, 20 adenovirus cell cultures were grown in A549 cell line until 2+ positive (25% of cell sheet) cytopathic effect appeared. These were then tested by the DNA probe and other methods for culture identification.

Results.

Out of 245 patients tested for adenovirus, 12 specimens were positive by culture, 10 each by EM and EIA, 14 by LA and in one patient by DNA probe (Table 3.5.1.). Two specimens were positive by LA that were negative by culture and 4 LA positive specimens failed to detect adenovirus by EM and EIA; two of these specimens were positive by cell culture. The LA method detected most positive specimens but it must be borne in mind that these may not all be true positives. Grandien et al. (1987) have shown that after excluding specimens also reacting with the latex control reagent, LA has a specificity of 100% but sensitivity of 85-90%. Interpretation of results can also be subjective.

Table 3.5.1. Comparison of culture, EM, EIA, LA and DNA probe in the detection of adenovirus from 245 acute diarrhoeal specimens.

	Adenovirus positive by				
Specimen #	Culture	EM	EIA	LA	DNA Probe
1	+	+	+	+	-
2	+	+	+	+	-
3	+	+	+	+	-
4	+	+	+	+	+
5	+	+	+	+	-
6	+	+	+	+	-
7	+	+	+	+	-
8	+	-	-	+	-
9	+	+	+	+	-
10	+	+	+	+	-
11	+	+	+	+	-
12	+	-	-	+	-
13	-	-	-	+	-
14	-	-	-	+	-
#15-#245	-	-	-	-	-
Total	12	10	10	14	1

Twenty CPE-positive cultures were also tested for culture identification by the different methods: 11 were detected by DNA probe, 18 by LA and 20 each by EIA and EM (Table 3.5.2). All 30 controls from normal healthy persons tested negative by all these methods, except for two specimens by LA which tested as non specific. The criteria used for non-specificity was agglutination occurring in the negative control.

Table 3.5.2. Detection of adenovirus in CPE-positive cell cultures by DNA probes and other methods.

	Number of patients	
Method	Tested	Positive
DNA probe	20	11
EM	20	20
EIA	20	20
LA	20	18

Since the DNA probe kit can only detect certain common adenovirus types (4,5,7,11,20,40 and 41) a further study was carried out in which 7 known positive types were grown in culture (2 of type 5, 2 of type 7, 2 of type 40/41, and 1 of type 4). These were tested by DNA probe and 4 of the 7 specimens were detected. Types 4, 40/41 were not detected.

Table 3.5.3. shows a comparison between various methods with respect to cost, time and ease of interpretation. The DNA probe was found to be the most expensive. It was also labour intensive due to the number of steps involved. EM and EIA were the most economical tests but the figures do not take into account the cost of special equipment. Culture and EIA required more time for sample preparation but EIA had a shorter incubation time. LA was the most rapid method. Cultivation of virus in cell cultures used the same comparative technologist time but required an incubation of at least 5-10 days.

Table 3.5.3. Comparative aspects of methods for identification of adenovirus.

Test	DNA probe	EM	EIA	LA	Culture
Inoculation time	15 min	15 min	20 min	5 min	20 min
Incubation time	1 hr	1 hr	1h 10min	3 min	5-10 days
Interpretation	Moderate/difficult	Easy	Easy	Easy	Easy
Special instrumentation	No	Yes	Yes	No	Yes
Cost(materials and labour)	$15	$7	$9	$9.5	$11.5

Discussion.

One of the relatively recent developments in the diagnosis of infectious agents has been the introduction of DNA probes. Various studies on commercially available DNA probes have shown sensitivities ranging from 31-94% for detecting infectious agents such herpes simplex and cytomegalovirus in CPE-positive cell cultures and direct detection in situ (Forghani et al., 1985; Langenberg et al., 1988; Qadri et al., 1988; Qadri et al.,1989). Although the DNA probe evaluated during this study contained the sequences for adenovirus type 40/41, the most frequent serotypes associated with diarrhoea, only 1 out of 14 specimens positive by LA was detected by the probe. Another study (Hyypia, 1985) showed that the colour of the stool samples caused difficulties in the interpretation of test results.

The DNA probe kit detects only 7 common adenovirus types, which may explain its low sensitivity, since these types are not necessarily the most prevalent in

different parts of the world. Also, the DNA probe did not detect all the adenovirus types it was designed for. It was also found that the hybridization step also sometimes damaged the cells making the result difficult to interpret.

The EIA and LA kits have common epitopes to detect all adenovirus types. Virus titres are sufficiently high in specimens such as stool to be detected directly with conventional methods as EM, EIA, and LA. However, it was found that the biotinylated DNA probe lacked the sensitivity for both direct antigen detection in stools as well as culture confirmation in CPE-positive cell lines. Other methods (EM, EIA, LA) proved to be superior. It was also found that the DNA probe was costly, labourious and difficult to interpret. In contrast, EIA and LA were simple to perform, sensitive and suitable for large scale screening.

The sensitivity of the DNA probe used during this study was too low for use as a reliable diagnostic test for adenovirus identification even after virus isolation. Although the manufacturer states that the kit was introduced for research purposes, and not for diagnostic use; these kits are frequently marketed and used in clinical laboratories as a diagnostic tool for rapid identification of the virus. Since DNA probes may require relatively high concentration of viral DNA in the sample to be tested, and this cannot be assured even after virus isolation in cell cultures, it is felt that the method is not suitable for reliable rapid identification in its present form

3.6 Diagnosis of Astrovirus by Reverse Transcriptase PCR in Stools.

Introduction.

There have been increasing reports of astrovirus-associated epidemics of gastroenteritis affecting both children and adults (Cubitt, 1990). Epidemiological studies on astroviruses have been limited because of the difficulties in establishing them in cell culture. Electron microscopy is used in diagnosis but is relatively insensitive and is not readily available to all laboratories. Hence, the prevalence of astroviruses may have been greatly underestimated. However, rapid developments in technology have made it possible for more convenient testing of clinical specimens. These include the polymerase chain reaction (PCR).

In order to evaluate reverse transcription PCR (RT-PCR) a published method (Major et al.,1992) was modified to determine the presence of astroviruses in diarrhoeal specimens from Saudi Arabia and England and compared with standard EM. Modifications were carried out to optimize the PCR method and included altering the concentration of dNTP and astrovirus primer. The extraction procedure was also modified as follows: GENETRON was used to resuspend the virus instead of Arcton 113, another detergent CTAB was used to break up the virus further and the aqueous layer was mixed with chloroform/isoamyl alcohol (25:1) and the virus precipitated in ethanol. In the original method, purified astrovirus was banded by CsCl isopycnic centrifugation and assayed by RNA dot-blot techniques.

Method.

Clinical Material. Stool specimens from 130 patients with diarrhoea at KFSH and RC and similarly 130 patients from Leicester U.K. were randomly selected for PCR and EM analysis. Twenty control specimens taken from food services staff and routine neonatal screens were also included. The positive control for RT-PCR was 1ng tissue culture-derived human astrovirus serotype 1 (kindly provided by Professor C.A. Hart, School of Tropical Medicine, Liverpool). Negative control was water.

Specimens for electron microscopy were filtered and the supernatant centrifuged at 37,400 x g for 1 hour at 4°C in a Beckman L7-55 ultra-centrifuge (Palo Alto, CA,USA). Grids (200 mesh formvar/carbon) were prepared by negative staining and examined on a Jeol electron microscope (Tokyo, Japan).

RNA extraction.

A 10% stool suspension was prepared and centrifuged (10K, 15 mins). 500 µl of stool supernatant was mixed with 500 µl of 1,1,2-dichloro-1,2,2-trifluoroethane (GENETRON) (Fisons, Loughborough, UK) and centrifuged (13K,15 mins). The top layer was removed followed by addition of PEG 6000 (final concentration 8%) and NaCl (final concentration 0.4M), which acted to concentrate the virus. The mixture was then allowed to precipitate for 30 mins at 4°C.

Following precipitation the mixture was centrifuged (13K,30 mins) and the supernatant discarded, leaving a pellet. To this, 500 µl of proteinase K buffer (0.1M Tris-HCl pH 7.5, 12.5 mM sodium EDTA, 0.15M NaCl, 1% SDS) was

added and the mixture incubated at 37°C for 1 hour. Cetylmethylammonium bromide (CTAB, Sigma, St. Louis,USA)(final concentration 1.25%) and NaCl (final concentration 0.45M) were next added and the mixture incubated again at 57 °C for 30 mins.

An equal volume of phenol/chloroform mixture (1:1) was mixed with 500 µl of mixture and centrifuged at 13K for 15 mins). The top layer was removed and mixed with an equal volume of chloroform/isoamyl alcohol mixture (24:1) and centrifuged (13K,15 mins). The upper layer was removed and mixed with 2.5 volumes 100% ethanol and nucleic acid was allowed to precipitate at -70°C for 1 hour. The ethanol was then removed after centrifuging at 13K for 30 mins. The pellet was resuspended with 50 µl of Tris-EDTA buffer (TE buffer).

RT-PCR.

Astrovirus primers as described by Major et al. (Major et al., 1992) were used to synthesize single-stranded cDNA (Astro 1 GCTTCTGATTAAATCAATTTTAAA, antisense and Astro 2 GATCCCTGCACATCTGGAAG,sense).

Fig. 3.6.1. Nucleotide sequence of the 3 end of astrovirus type 1. The underlined sequences indicate the regions used for construction of synthetic oligonucleotides for PCR amplification. The sequence is numbered from the poly (A) tail towards the 5 end. (Major et al., 1992).

```
      -110        -100        -90         -80         -70         -60
ATCCCTGCACATCTGGAAGCCGCGGCCACGCCGAGTAGGAACGAGGGTACAGCTTCCTT

        -50         -40         -30         -20         -10
CTTTTCTGTCTCTGTTTAGATTATTTTAATCACCATTTAAAATTGATTTAATCAGAAGC(A)n
```

The following mix was used to reverse transcribe genomic RNA: 5 µl 10x RT buffer (550 mM KCl, 300mM Tris, 40 mM $MgCl_2$, 30mM dTT) (Cambio, Cambridge, England), 0.4 mM each nNTP, 0.2 µg/ml gelatine, 50 ng primer astro 1, 5 units MoMuLV-RT (Cambio, Cambridge, England), 10µl RNA extract. The total reaction volume was 50 µl. This mix was incubated at 42°C for 30 mins before being stopped by heating to 94°C for 2-5 mins. 5 µl of cDNA was amplified in a reaction buffer containing 55 mM KCl, 30 mM Tris-HCl (pH 8.3), 4 mM MgCl, 3mM dithiothreitol, 20 Mm of each dNTP, 0.2 mg/ml gelatine, 0.04 mg of each primer and 5 units of Stoffel Taq polymerase (Perkin-Elmer, Norwalk,USA). The PCR amplification was 30 cycles at 93°C for 1.5 mins, 55°C for 1.5 mins, 74°C for 1.5 mins. The amplified DNA product was analysed on a 2% agarose gel and visualised by ethidium bromide staining with ultraviolet light illumination.

Results.

Stool specimens from 260 patients were tested for the presence of astrovirus along with 20 patient controls. Of the 130 patients with diarrhoeal illness in each centre, 53 were aged 0-5 years, 24 aged 6-19 years, 44 in the 20-55 age group and 9 over the age of 55 years. All the control stools tested negative and only two Saudi specimens were found to be positive in the test samples by PCR (1.5%). One of the positive specimens was from a 5 year old female with rotavirus infection and presenting with both diarrhoea and vomiting. The second was from a 5-month old female in which a small round virus, presumed astrovirus, had been observed by EM. None of the samples had astrovirus diagnosed by electron microscopy. Of the 130 Leicester specimens, 21 were positive for astrovirus by PCR. Only 16 of these had astrovirus detectable by EM. One dual infection with rotavirus was found.

Discussion.

Infection with astrovirus has been associated with gastroenteritis in both children and adults, and serological surveys indicate that this infection may be frequent (Kurtz and Lee, 1978; Lee and Kurtz, 1994). Studies in some developing countries have shown prevalence rates of 1.5% in Nigeria (Avery et al., 1992), 1.2% in Malawi (Pavone et al., 1990), 4.8% in Brazil (Sttewien et al., 1991), 7.3% in Guatemala (Cruz et al., 1992), and 8.6% in Thailand (Hermann et al., 1991). In the U.S.A. and U.K. the prevalence rate has been found to be approximately 4%

(Kurtz and Lee, 1978; Lew et al., 1991). In this study the figure was 1.5% in Saudi Arabia, 4.6% in Leicester, England. The discordance of the prevalence results may however, have another explanation. This PCR method is based on detecting sequences at the 3' end of the genome. These sequences appear to be specific for astrovirus serotype 1, and it may be that, although most isolates in the U.K. are of this serotype, another predominates in Saudi Arabia. Another possibility may be that Saudi Arabia is a very large country in which communities are more isolated resulting in less spread of virus.

Standard EM was found to be relatively insensitive method since greater than 10^6 particles are required for detection and a skilled electron microscopist is required to differentiate SRSVs. However, it does have the advantage that many non-cultivatable viruses can be examined for at one time. However, PCR is sensitive and potentially a more specific assay. PCR can detect minute amounts of RNA and given its rapidity and reproducibility can allow for routine detection of astroviruses. Nested polymerase chain reaction methods have now been described which may have even greater sensitivity and specificity (Jonasson et al., 1993). This increase in sensitivity may have to result in caution in making an aetiological diagnosis, however, as the two dual infections found in this study had astrovirus at a level detectable only by RT-PCR not EM.

The source of infection of astroviruses and other SRSVs is thought to be polluted water and contaminated seafood (Le Baron et al., 1990). This may explain the relatively low figure in Saudi Arabia study due partly to the higher socio-economic

patient population examined and being a hot desert area where most water is received from desalination plants. Person to person spread has also been shown to be responsible in children where there are poor hygiene practices.

Present knowledge of astrovirus has shown it to be a self-limiting disease except in immunocompromised hosts where it can cause chronic diarrhoea and prolonged shedding. Infection with astrovirus is also more significant when it occurs in conjunction with other enteric agents when oral rehydration may be necessary, this was found to be the case in one of the positive patients. However, the socio-economic impacts are particularly significant and new rapid tests will be beneficial in stemming transmission via contaminated food, reducing nosocomial infections and determining the true epidemiology of astrovirus infections.

3.7. Other Enteric Viruses Isolated at KFSH & RC.

Due to the changing trends between parasite host relationships, other viruses that have been implicated in causing diarrhoea were also examined in order to assess the extent to which these viruses are involved.

3.7.1. Cytomegalovirus.

Introduction

Cytomegalovirus causes severe infections in congenitally infected infants, and immunocompromised patients. In addition to pneumonitis, hepatitis, and renal

graft dysfunction; patients with severe or fatal CMV infection may develop colonic ulceration. The evidence for CMV as an aetiological agent in enterocolitis is best provided by endoscopy or EM examination of tissue sections since isolation from the gut may be due to the prescence of blood.

Method.

Specimens for CMV isolation were set-up for both shell vial assay and conventional culture. In the shell vial assay, human foreskin cells were grown on a coverslip in dram vials. 0.3 ml of processed specimen was inoculated in duplicate and centrifuged for 40 min at 700 x g. The specimen was removed to prevent toxicity and 1 ml of maintenance medium L-15, containing foetal bovine serum , glutamine and antibiotics was added to each tube. One tube was read after 24 hours and the other at 48 hours using an indirect monoclonal antibody (Syva, Palo Alto, Ca). Conventional cultures were set up using diploid fibroblast cell line and monitored for 28 days for positive CPE.

Results.

Over a 12 month period a total of 70 patients were found to be positive for CMV from all specimen types. Of these there were 4 patients that had enteric CMV infection.. These were all found in immunocompromised adults and all had symptoms of diarrhoea (Table 3.7.1.1.).

Table 3.7.1.1. Clinical diagnosis of patients with enteric CMV infections at KFSH & RC.

Age	Sex	Primary Diagnosis	Secondary diagnosis	Specimen	Sigmoidoscopy
24	F	liver transplant	ulcerative colitis vomiting, diarrhoea	rectal biopsy	yes
37	M	liver transplant	chronic diarrhoea	small bowel tissue	yes
26	F	chronic lymphocytic leukaemia	vomiting, diarrhoea	oesophageal biopsy	yes
58	M	Renal transplant	diarrhoea (more than 15 days)	rectal tissue	yes

Discussion.

The management of infection in the immunocompromised host is a difficult and continuing problem. CMV infections are being diagnosed more frequently in immunosuppressed patients and with the development of an effective drug therapy their rapid recognition has assumed greater clinical importance. CMV is a potentially serious pathogen of the gastrointestinal tract in HIV-1 infected people and transplant patients. CMV colitis is characterized by diarrhoea, fever, and weight loss. Also CMV can cause disease involving any region of the gastrointestinal tract. Gastrointestinal complications of CMV infection, although

rare, in immunocompetent patients can be life threatening and often require extensive surgery (Consten et al., 1993).

3.7.2. Enteroviruses

The enteroviruses comprise over 70 serotypes which have been isolated from man. Their normal site of replication is the intestinal tract, where infection may be clinically inapparent or result in a mild gastrointestinal disorder. In the proportion of cases the virus spreads to other organs causing severe illnesses.

Results

Over a 12-month period a total of 554 specimens were received for stool culture. These were set up in A549, RMK, HFS and LLC-MK2. Of these 19 were found to be positive for enteroviruses by CPE and the indirect immunofluorescent test (Dako). These isolates were typed by neutralizing antisera (Lim-Benyesh Melnick typing pools-WHO). Polioviruses isolated were most likely due to vaccination rather than wild types since they were detected in children.

Table 3.7.2.1. Enterovirus serotypes identified at KFSH & RC.

#	Age	Sex	Enterovirus type
1	1y 6m	F	coxsackie B2
2	1y 6m	F	coxsackie B3
3	5y	M	coxsackie B3
4	1y	M	coxsackie B4
5	2y	M	coxsackie B5
6	5y	F	echovirus E2
7	1y 4m	M	echovirus E6
8	2y	F	echovirus E17
9	7m	M	echovirus E17
10	7m	M	echovirus E17
11	1y 2m	M	echovirus E19
12	1y 7m	M	echovirus E19
13	1m	F	echovirus E30
14	7m	M	echovirus E30
15	4y7m	F	poliovirus type1
16	2m	M	poliovirus type2
17	2m	M	poliovirus type 3
18	7m	M	enterovirus
19	11y	M	enterovirus

Discussion.

Enteroviruses can cause mild gastrointestinal disorders and are commonly reported among associated symptoms in which other clinical features predominate. In many outbreaks, the enterovirus may be a passenger virus unrelated to the illness itself. However, some enteroviruses particularly echoviruses 4, 11, 14, 18 and 19 have

been documented in relation to outbreaks of diarrhoea (Kibrick, 1964).

In an outbreak of gastroenteritis in bone-marrow transplant recipients, coxsackie A1 was isolated from all 7 patients with diarrhoea, constituting 50% of the patients in the unit during a three week period (Townsend et al., 1982). A further prospective study of 78 patients on the same unit showed virus isolation from 22 patients, some of whom were doubly infected (Yolken et al., 1982). At this hospital most of these infections were found in children under 5 years and echo-viruses predominated. Although enteroviruses play a minor role as aetiological agents of gastroenteritis, they may play a a more significant role in immunocompromised patients.

3.7.3. Coronaviruses

Coronaviruses are pleomorphic, enveloped RNA-containing viruses. Coronaviruses are known to cause severe gastrointestinal disease in animals but their role in human gastroenteritis is controversial. However, there are two case-control studies which suggest that a relationship does exist between coronaviruses and gastrointestinal disease (Vaucher et al., 1982; Gerna et al., 1985).

At this institute 3,000 diarrhoeal specimens were examined by EM. of which only 8 cases of coronavirus were observed. Coronavirus is the only membraned virus that is occasionally seen in stools. It is pleomorphic measuring 80-130 nm in diameter, it differs from the myxoviruses in that its spikes are long and clubshaped (Miller 1986). Taking

into account their very low prevalence and their controversial role in gastroenteritis it was considered that they do not play any significant role in the Saudi population.

3.8. Diagnosis of viral gastroenteritis at two medical centers inSaudi Arabia and the United Kingdom.

Introduction.

Rotaviruses are responsible for 50% of the gastroenteritis in hospitalized paediatric patients in countries with temperate climates. But the prevalence in adults and children have been found to be very low in rural areas and has been attributed to less overcrowding, different diet and gut flora (Garthwright et al., 1988). The prevalence rate of enteric adenoviruses has also been found to differ in different parts of the world. It is the second most common virus detected in the U.S.A., U.K., and Scandinavia (Flewett et al., 1975; Brandt et al., 1979; Brandt et al., 1985), the third most common virus in India (Maiya et al., 1977) and Canada (Middleton et al., 1977) and the fourth most common virus in Scotland (Madeley et al., 1977). However, epidemiological studies in Thailand have shown astrovirus to be the second most common viral pathogen (Herrmann et al., 1991).

In order to determine the differences in prevalence of enteric viruses, a comparative study was carried out between a tertiary care facility in Saudi Arabia and a large medical center in the U.K.. These are two contrasting regions in terms of climate and patient population. Stored data over a three year period from both centres was examined.

Method.

KFSH & RC is a major referral center for Saudi Arabia and the Middle East.

Leicester Royal Infirmary (LRI) is a large teaching hospital serving a base population of one million people in Central England. Rotavirus was performed by EIA and performed according to manufacturer's instructions. Examination for diagnosis of adenovirus and SRVs was performed by electron microscopy. LRI used EM for the detection of all viruses in stool specimens.

Results.

A total of 3400 patient specimens at LRI and 2980 patients at KFSH &RC were examined during 1990-1992. Rotavirus was the most common viral pathogen detected at both centers with adenovirus second. However, the incidence of rotavirus at LRI was 14.6% during this period and 11% at KFSH & RC. SRVs were not further characterized at KFSH & RC but at LRI they detected 24 astroviruses, 10 caliciviruses and 42 other SRVs. Nevertheless, the prevalence rates at both centres were similar.

Table 3.8.1. Comparison of viral gastroenteritis diagnosed at KFSH and RC and LRI between 1990 and 1992.

	LRI			KFSH		
	1990	1991	1992	1990	1991	1992
Rotavirus	177 (16%)	180 (16%)	141 (12%)	66(8%)	111(11%)	151(13%)
Adenovirus	48 (4%)	54 (5%)	31 (2.5%)	17(2%)	15 (1.5%)	47 (4%)
SRVs	39 (3.5%)	19 (2%)	18 (1.5%)	- -	10 (1%)	23 (2%)
Total	1100 (100%)	1100 (100%)	1200 (100%)	826(100%)	990(100%)	1164(100%).

Discussion.

The detection of viral agents has become increasingly important in terms of prophylaxis, quarantine of patients and better patient management. Even in industrialized countries rotavirus diarrhoea in the young is among the leading causes of hospitalization. In temperate regions institutional outbreaks of the disease occur mainly in cold dry weather, whereas in tropical settings the seasonality is less well defined (Konno et al., 1983). At KFSH & RC the majority of rotavirus infections were found in children less than one year old (60%) although, most infections in neonates are asymptomatic possibly due to protection afforded through breastfeeding (Frayh et al., 1987). There was no significant seasonal variation with only a slight increase between October and January, the colder months in Saudi Arabia.

Enteric adenovirus infections tend to be endemic rather than epidemic, although outbreaks in hospital nurseries do occur. Adenovirus infections and symptoms usually occur in children up to the age of three (Kidd et al., 1982). A conscientious examination for adenoviruses in stools was started in 1992 and initially there was found to be a prevalence rate of 4% with over 70% of infections occurring in children under 6 years of age.

Epidemiologic studies of SRVs and SRSVs have been limited due to a lack of adequate viral detection and serologic assays. However, some studies have shown that in less developed countries such as Bangladesh, most people have Norwalk

virus antibodies by the age of 5, whereas in the U.S.A. acquisition of antibodies occurs over the first two decades of life. This suggests that transmission may occur by the faecal-oral route and is dependent on sanitary conditions (Greenberg and Matsui, 1992). Antibodies to other SRSVs such as astroviruses also occurs in early childhood. A study in the U.K. showed that 70% of children had antibodies by the age of 5 and 75% by the age of 10 (Lewis et al., 1993). Identification of SRSVs and SRVs has been difficult due to the expertise required in differentiating them. LRI have qualified and experienced microscopists since EM is their mainstay method in diagnosing diarrhoeal specimens. However, this is a subjective method and with the advent of new methods such as RIA, EIA and PCR, identification of these agents will become available to more laboratories.

In another parallel study of gastroenteritis in the U.K. and Nigeria, rotavirus was found in 26% of specimens in Nigeria and 22% in the U.K.. However, in Nigeria there were no enteric adenoviruses detected and only 1.5% astroviruses compared to 13.6% adenoviruses and 4.5% astroviruses in the U.K. (Avery et al., 1992). It is unlikely that these are realistic figures since only 66 stool specimens were examined in the study. Increased awareness of the aetiology of viral gastroenteritis at KFSH & RC resulted in a greater number of positive identifications over the study period. The observation that incidence of rotavirus was lower than at LRI may be due to less overcrowding and the lower numbers of specimens examined, although this did not apply to adenoviruses and SRVs.

Despite the advances in rapid detection and diagnosis of viral gastroenteritis, the prevalence rates of these agents remain high in both industrial and developing countries. This indicates the need to combat the spread of these viruses through vehicles such as water, fomites, food, and especially nosocomial transmission of infection. Such measures may further help to contain these relatively resistant and ubiquitous agents.

Chapter 4. Transmission Studies of Enteric Viruses.

4.1. Viral contamination of environmental surfaces on a general paediatric ward and playroom in a major referral center in Riyadh.

Introduction.

Nosocomial infections are a well recognized cause of morbidity in hospitals and day-care centres where they provide a situation in which the potential spread of infectious diseases is increased. Environmental contamination can play a significant role in transmission. This is particularly true for infants who frequently place contaminated objects and fomites in their mouths. In the literature, the majority of cases of nosocomial diarrhoea (56%) have been transmitted by indirect transmision i.e. hospital personnel or contaminated environmental fomites, 22% of cases were caused by direct person-to-person transmission, and in another 22% the route of transmission has not been determined (Ansari et al., 1991).

Environmental contamination by faecal coliforms is common and contamination has been shown to increase during outbreaks of diarrhoea (Bean et al., 1982; Ekanem et al., 1983). Many viruses have also been found to survive in the environment and rotavirus in particular has been described as a frequent cause of

nosocomial diarrhoea. Rates of 17% have been reported on infant wards (Flewett, 1981; Giannella, 1993). Rotavirus can be transmitted to previously unexposed children and to family contacts, including adults due to close proximity. Challenge studies have shown the minimal infective dose to be as little as one cell culture infective unit (Hall et al., 1980).

Environmental surfaces can also be contaminated with respiratory syncytial virus (Ijaz et al., 1985), influenza A,B (Lam et al., 1989), and rhinoviruses through contact with contaminated hands and by settling of aerosolized nasopharyngeal secretions. Infectious virus has been recovered in fomites from people with rhinovirus colds (Keswick et al., 1983). Other viruses such as Hepatitis A can cause outbreaks of disease in hospitals and have been shown to survive for several days at ambient temperatures.

There have been no such data available to date in Saudi Arabia. Therefore, a 6-month prospective study was carrried out to investigate the presence of viruses on environmental surfaces.

Method.

The site chosen for investigation was a general paediatric ward and playroom. The ward had chronic patients with pneumonia, fever, cardiac problems, enteric infections etc. and approximately two weeks stay or longer. The playroom is at the entrance to the ward. Swabs were taken weekly from non-porous sites such as plastic toys, countertops, washrooms, and other fomites. Non-porous sites were not

sampled and may have caused underestimation of viruses but this was due to difficulties of specimen collection. Patient and employee hands were also sampled prior to washing by swabbing the palms of both hands. An area approximately $4cm^2$ was taken from each site. The study was carried out between August 1993 and February 1994.

Specimens were collected in virocult swabs and inoculated into a mixture of tryptose-phosphate broth with 0.5% gelatin and antibiotics. 0.1 ml was then inoculated into the following cell lines: primary rhesus monkey kidney (RMK) for the detection of influenza and parainfluenza; human lung carcinoma (A549) for the detection of adenovirus and herpes simplex; human foreskin (HFS) for the detection of cytomegalovirus and rhinovirus; and secondary rhesus monkey kidney (LLC-MK2) for the detection of enterovirus. All cultures were kept for two weeks and examined for cytopathic effect on alternate days. CMV cultures were kept for 21 days. All tubes were fed every 4 days with fresh medium. The haemadsorption test was carried out on RMK after 5 days and on LLC-MK2 after 10 days to detect myxo- and paramyxoviruses. EIA methods were also used for the detection of rotavirus and enteric adenoviruses (type 40/41). The EIA method has been shown to be specific and is 2 $\log_{10}$ more sensitive than electron microscopy (Mbithi et al., 1993).

Results.

Rotavirus was detected in 11 out of 155 specimens sampled from environmental surfaces (7%). It was found that the detection of rotavirus on environmental

surfaces correlated with the occurrence of rotavirus infection in patients (Table 4.1.1). Rotavirus was detected on surfaces that involved the most human activity such as toilet handles, televisions, vital signs charts and toys (4.1.2.). One patient carried rotavirus on the hands but no staff had rotavirus on the hands. No other viruses were recovered from the environment despite the fact that several respiratory viruses were recovered from patients during this period (Fig 4.1.3). The temperature in the ward and out doors was recorded each week and an increase in prevalence of rotavirus was noted between October to January when the lowest temperatures occurred (4.1.4.).

During the month of April, 7 patients on the ward were found to be positive for rotavirus and a further 22 specimens were collected during this period to determine whether there was any increased colonization of rotavirus in the environment. Two out of 22 specimens sampled were found to be positive. One positive sample was from the soiled clothes of a patient with rotavirus and the second from a telephone in the same room.

Table 4.1.1. Location of Rotavirus from Patients and Environmental Surfaces.

Month	No.	Positive Rotavirus in environment	No.	Positive Rotavirus in patients
August	0		0	
September	0		0	
October	3	bed sink toilet handle	3	12 months female 4 months male 36months female
November	3	toy baby chair vital sign chart	2	6months male 7months female
December	2	Box of toys handle patient hand	1	12months female
January	1	toilet handle	1	8months male
February	2	television drawer	0	

Table 4.1.2. Sites of Environmental Surfaces.

Surface	No. specimens taken	No. positive rotavirus	No. of samples in outbreak	No. positive in outbreak
Toys	34	2	5	-
Furniture	44	4	2	-
Medical equipment	8	-	1	-
Door handles	6	-	1	-
Telephones	9	-	3	1
Vital signs chart	6	1	1	-
Toilet handles	9	2	1	-
Patient hands	21	1	3	-
Doctor/Nurse hands	6	-	1	-
Sinks	6	1	1	-
Misc-bottles, flasks, clothes	6	-	3	1
Total	155	11 (7%)	22	2 (9%)

Fig. 4.1.3. Viruses isolated on a Paediatric Ward Aug 1993 - Feb 1994.

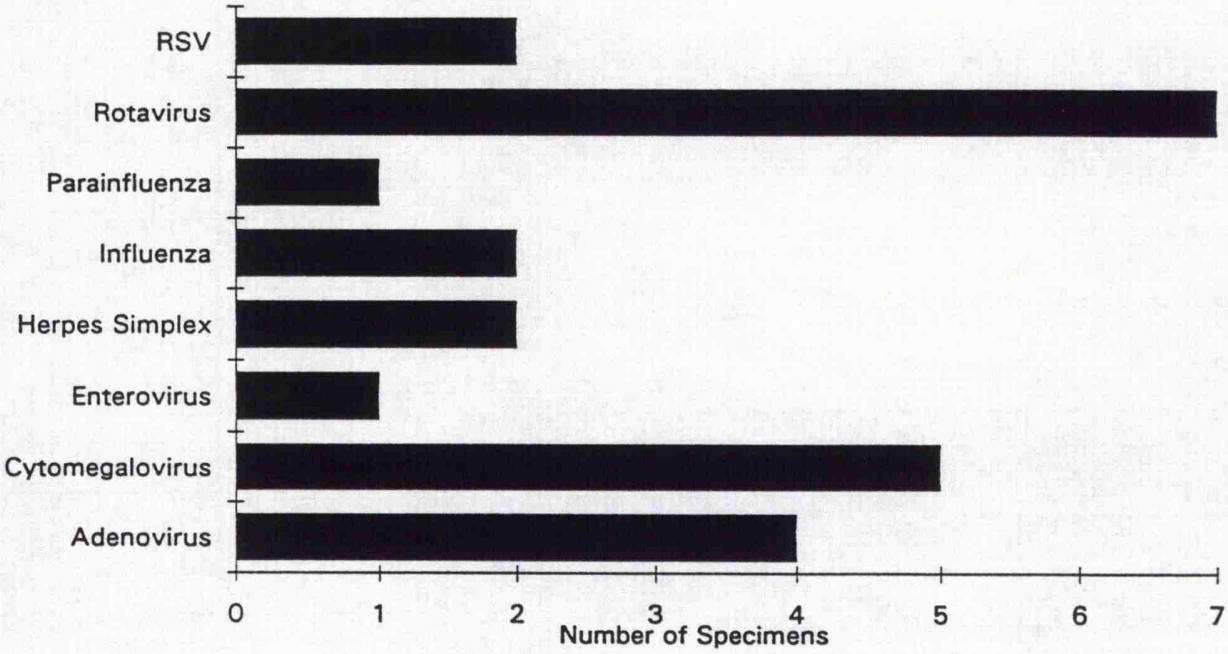
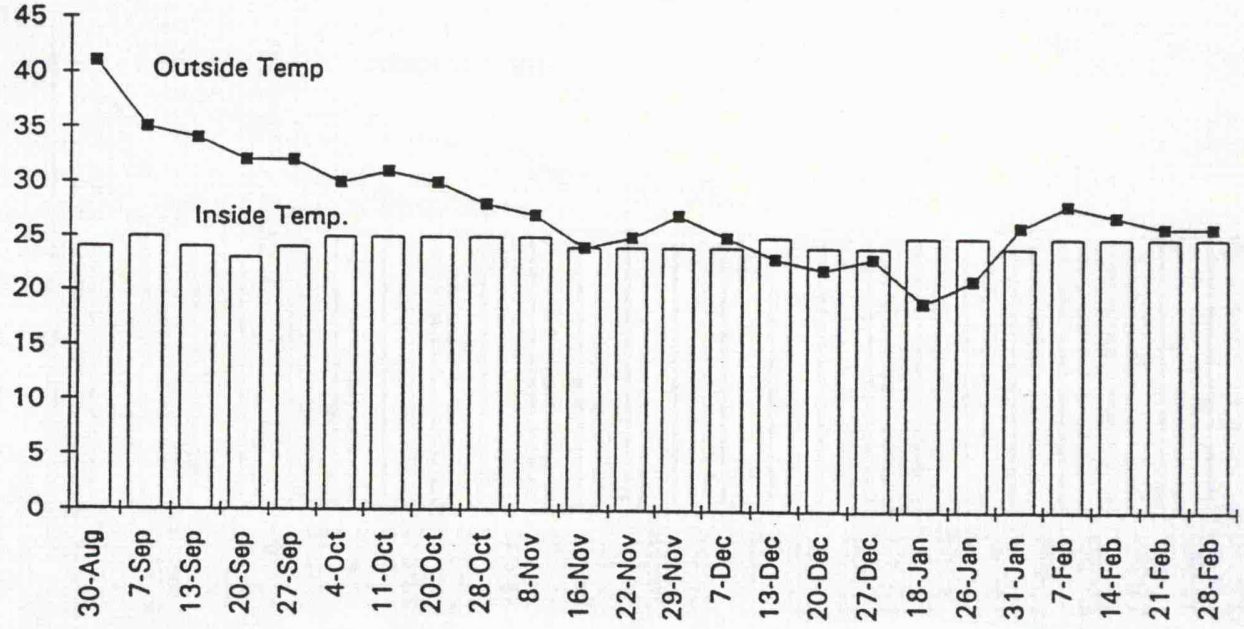


Fig. 4.1.4. Internal and External Temperature Readings on a Paediatric Ward between Aug 1993 - Feb 1994.



Discussion.

The environment in hospitals and day care centers can be heavily contaminated with viruses and bacteria. This was demonstrated by Keswick et al. (Keswick et al., 1983) who sampled 25 sites in a day care center from employees and teachers hands; they found 4 positive for rotavirus (16%). In another study, Zavate et al. (Zavate et al., 1980) reported 6% of samples collected from objects in a child care facility were contaminated with enteroviruses. These studies suggest that diarrhoea-causing micro-organisms may be commonly present on environmental surfaces and transmitted to susceptible children. In this study 7% of samples had rotavirus.

Nosocomial viral infections can greatly add to costs of hospitalization and prolonged hospital stay (Ryder et al., 1977). This is increasingly important in paediatric tertiary care centers, due to the increasing number of compromised patients (cancer, organ transplant, immunodeficiency) who experience considerable morbidity and mortality from such viral infections as rotavirus diarrhoea. Rotaviruses are a leading cause of diarrhoea in children and has been estimated that 10^{10} particles per gram of faeces may be shed by children with diarrhoea (Sattar et al., 1993). They are viable for up to 25 days on surfaces and 7 months in storage at room temperature (Sattar et al., 1989).

A wide range of viral infections were diagnosed in patients on the paediatric ward. However, only rotavirus was detected on environmental surfaces. Other viruses

may be unable to survive the hot, dry conditions that exist in Saudi Arabia. A dry atmosphere, in combination with lower air temperature appears conducive to outbreaks of rotavirus. This would agree with our observations where an increase in rotavirus contamination was noted between October-January when the lowest external tempertures were recorded. The presence of rotavirus in the environment also coincided with rotaviral diarrhoea in patients. The relative humidity in the ward is maintained at approximately 40% which may also be conducive to virus survival. Ijaz et al. (1985) showed that at 20°C and 50% humidity, aerosolized rotavirus from faeces remained infectious at 24 hours. During the increased incidence of rotavirus infection on the ward one patient area showed increased rotavirus colonization. Not all patient areas were found to be colonized, this may be due to poor hygiene or poor cleaning practices.

Investigation of cleaning practices showed that only general purpose cleaners were used to clean all surfaces and no disinfection took place. Studies have shown that use of a chemical disinfectant such as lysol can reduce infectivity by 99.9% (Tan et al., 1981). Disinfection alone is insufficient to reduce viral titres since only a small dose is required for infection (0.9 focus forming units). However, washing can significantly reduce titres by physical removal and followed by disinfection may be more effective in reducing titres to a non-infectious level. However, due to the ubiquitous nature of rotavirus, development of an effective vaccine may provide the best solution.

Solutions containing organic iodine, hypochlorite or quaternary

ammonium salts as the principal active ingredient(s) are ineffective in inactivating rotavirus. Phenol-based products give variable results but products containing 60% ethanol or organic acids are able to inactivate the virus (Ward et al., 1984; Ward et al., 1986; Weniger et al., 1983). Medicated hand washing agents can also greatly reduce viral titres (92% reduction) and are far superior to non-medicated hand washing agents (Zavate et al., 1980). Hence, a combination of such measures would be more effective in removing viruses from the environment. Also, toys are often overlooked and left uncleaned for extended periods of time. Soaking toys in disinfectant at the end of each day would remove this potential reservoir of infection.

Rotavirus can survive on human hands for several hours and so decontamination of hands alone may not be sufficient to prevent spread of virus. The wearing of gloves and gowns and a programme of proper and regular disinfection of environmental surfaces may play a significant role in reducing spread of nosocomial infections. Increasing the relative humidity to 80% results in a rapid loss of infectivity and may be another possible mode of decontaminating isolation rooms where cases of diarrhoea occur (Yolken et al., 1981).

An attempt was made to electropherotype the positive specimens using polyacrylamide gel electrophoresis. However, there were insufficient titres of virus to appear as bands. Only one specimen showed up on the gel and exhibited the long electropherotype pattern that is predominant at this institute.

Before this study the nature of virus survival in hospitals in Saudi Arabia was not available. This report shows that rotavirus can readily survive on environmental surfaces as in other parts of the world. However, the absence of other viruses may require more sensitive methods for detection such as PCR and DNA probes, before the true prevalence and significance of viral load is known. In developing countries viral gastroenteritis is a significant cause of morbidity due to overcrowding and poor sanitation. It is almost certain that insects can also mechanically spread rotavirus under these conditions. The survival capacity of rotavirus in the environment requires close monitoring and education of staff as well as patients in proper handwashing and personal hygiene is necessary to eliminate this mode of transmission.

4.2. Electropherotyping of Rotavirus infections in patients at a tertiary care facility.

Introduction

Human rotavirus is a major aetiological agent of infantile gastroenteritis. Group A rotavirus is known to be the most common cause of severe diarrhoea among children in North America and Europe. A new group of rotaviruses (group B) has been found to cause large outbreaks of severe diarrhoea in China but has not been widespread outside of China (Hung, 1984). Rotavirus infects virtually every child in the U.S.A. by the age of 4 years and causes potentially lethal dehydration in 0.75% of children less than 2 years of age (Le Baron, 1990). The problem of acute gastroenteritis in developing countries is of much greater magnitude where 5 million deaths occur annually in Africa, Asia and Latin America alone (Snyder and Merson, 1982). Approximately one million of these deaths are due to rotavirus (Wild, 1991).

Group A rotaviruses has three major antigens, the subgroup antigen, the VP7 serotype antigen, and the VP4 serotype antigen. The rotavirus genome consists of 11 double-stranded RNA segments and at least two distinct subgroups I and II. Seven different VP7 serotype antigens have been distinguished so far (Kapikian, 1990).

Rotaviruses are difficult to cultivate and characterize. This has hampered

understanding of the epidemiological features of individual serotypes prevailing worldwide. This is particularly true of developing countries where it is necessary in order to develop an effective vaccine. Hence, molecular techniques such as the analysis of the electrophoretic mobility of the 11 double-stranded RNA segments of rotavirus by polyacrylamide gel electrophoresis (PAGE) are now being used for epidemiological studies (Estes 1984). This is useful in determining the different electropherotypes circulating in different geographic areas. They can be classified as L (long) or S (short), depending on the migration of segments 10 and 11. Rotavirus strains with short electropherotypes have been found to be associated with subgroup I and serotype 2 and 8, whereas strains with long electropherotypes are associated with subgroup II and serotypes 1,3,4 and 9.

This is the first report of electropherotyping carried out in Saudi Arabia at King Faisal Specialist Hospital and Research Centre.

Materials and Methods.

RNA genome analysis was performed on 200 rotavirus specimens positive by enzyme-immunoassay and/or electron microscopy (EM). The EIA detected only group A rotaviruses and EM detected both group A and non-group A rotaviruses.

Extraction

Extraction was carried out by the method of Herring et al. (1982). Briefly, double stranded genome of rotavirus present in stool specimens was extracted as follows. A heavy suspension of faecal material was suspended in 0.5ml of sodium acetate buffer (pH 5.0) containing 1% sodium dodecyl sulphate. To this was added an equal volume of phenol:chloroform: isoamyl alcohol (25:24:1). The mixture was shaken vigorously in a 1.5 ml eppendorf tube for 1 minute on a vortex mixer. This was followed by centrifugation at 6,000 rpm for 5 min. The clear upper layer containing double stranded RNA was removed and a 40µl aliquot was mixed with 15 µl of loading buffer (0.5 M Tris (pH6.8), 25% glycerol, 0.25% bromophenol blue).

PAGE

Electrophoresis was carried out in a slab polyacrylamide gel with SDS omitted from all buffers. An 8% polyacrylamide gel was used and 50 µl of each RNA preparation was carefully loaded into each well. Electrophoresis was carried out at room temperature at a constant voltage of 70V. All solutions were made from sterile distilled water and degassed for 15 minutes before use.

Silver staining.

The gels were washed with 10% ethanol-0.5% acetic acid for 30 min and then soaked in 0.011 M silver nitrate for 2 hours. The gel was washed in

distilled water and the reduction step performed with a solution of 0.75 M sodium hydroxide containing 0.1M formaldehyde and 0.0023 M sodium borohydride. Once the bands were visible the gels were placed in a solution of 5% acetic acid for 30 min followed by 0.07M sodium carbonate.

Classification.

To facilitate systematic comparison of the viral RNA patterns encountered, the method of Lourenco et al. (Lourenco 1981) was used. In this method 11 RNA segments once resolved were divided into 4 groups and electropherotypes identified as combinations of the variations within each group.

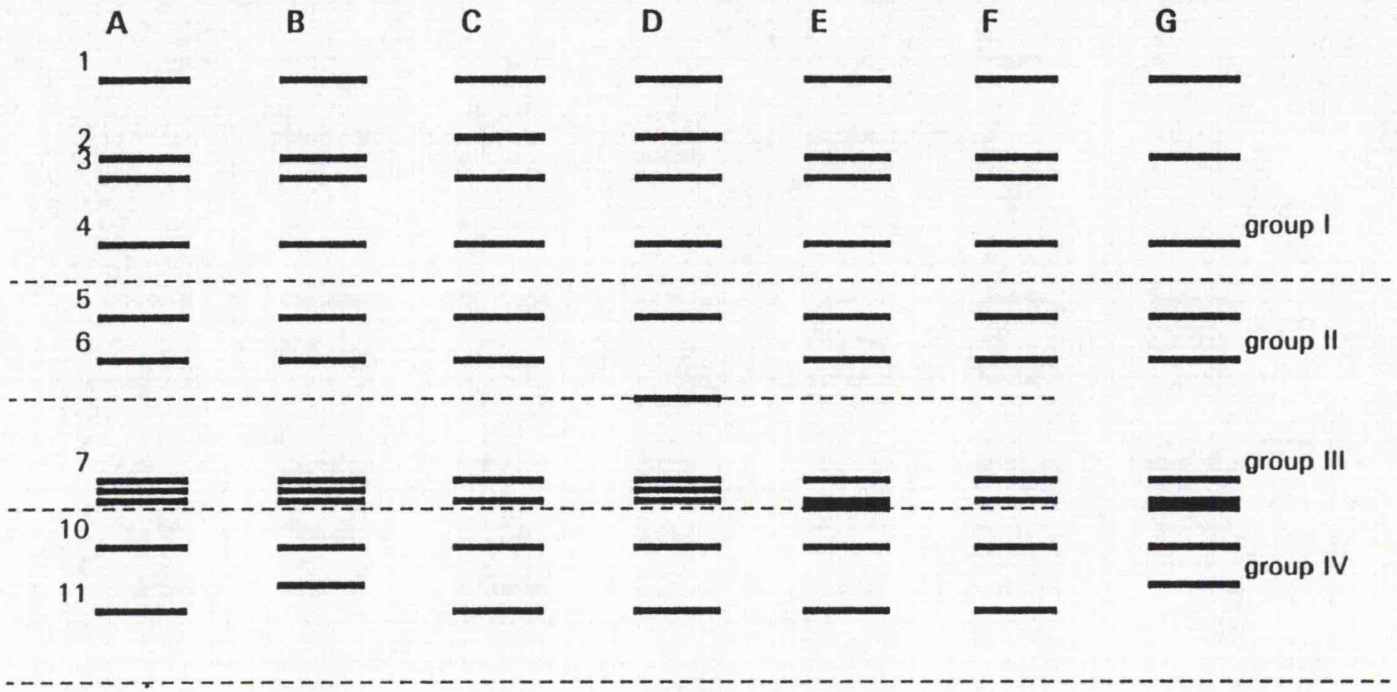
Results.

Electropherotypes of 200 isolates collected at King Faisal Specialist Hospital and Research Centre revealed 7 patterns of which 5 were long and 2 short (Fig 4.2.1). One of the long electropherotypes (A) dominated for the whole investigation period. No specific electropherotype correlated with patient age. Mixed infection was also identified. Facilities for taking photographs were not available and so gels were wrapped in plastic film and photocopied (Fig 4.2.2.). The greatest variations in mobility were found in segments 2,3,6, 8 and 9; segments 1,4, and 10 showed most unchangeable mobility. The distribution of electropherotypes showed there were 159 with pattern A, 21 with B, 3 with C, 6 with D, 2 with E, 2 with F and 3 with pattern G.

Analysis of patients demonstrated that 75% of infections occurred in children 5 years or less. A significant number of infections were also found in children under 1 year old (33%) due in part to the greater number of premature babies and immunocompromised patients at this hospital. A seasonal variation was observed with more infections occurring in the cooler months .

This simple method was found to separate rotaviral RNA bands. However, it was found that by increasing the current to 200 mA separation of bands could be improved. Using this modification gels could be run for 5 hours and stained the same day. Any increase of current above this caused overheating and buckling of the gels. Use of a 1mm thick gel instead of a 1.5 mm thick gel also improved the separation of the bands slightly. Initially gels were produced with a 3% stacking gel, however, this did not offer any advantages and so a continous 8% gel was used subsequently, which also improved the rapidity of the results (Fig 4.2.3.).

Fig. 4.2.1. Schematic patterns of migration of rotavirus electropherotypes in diarrhoeal patients in Saudi Arabia.

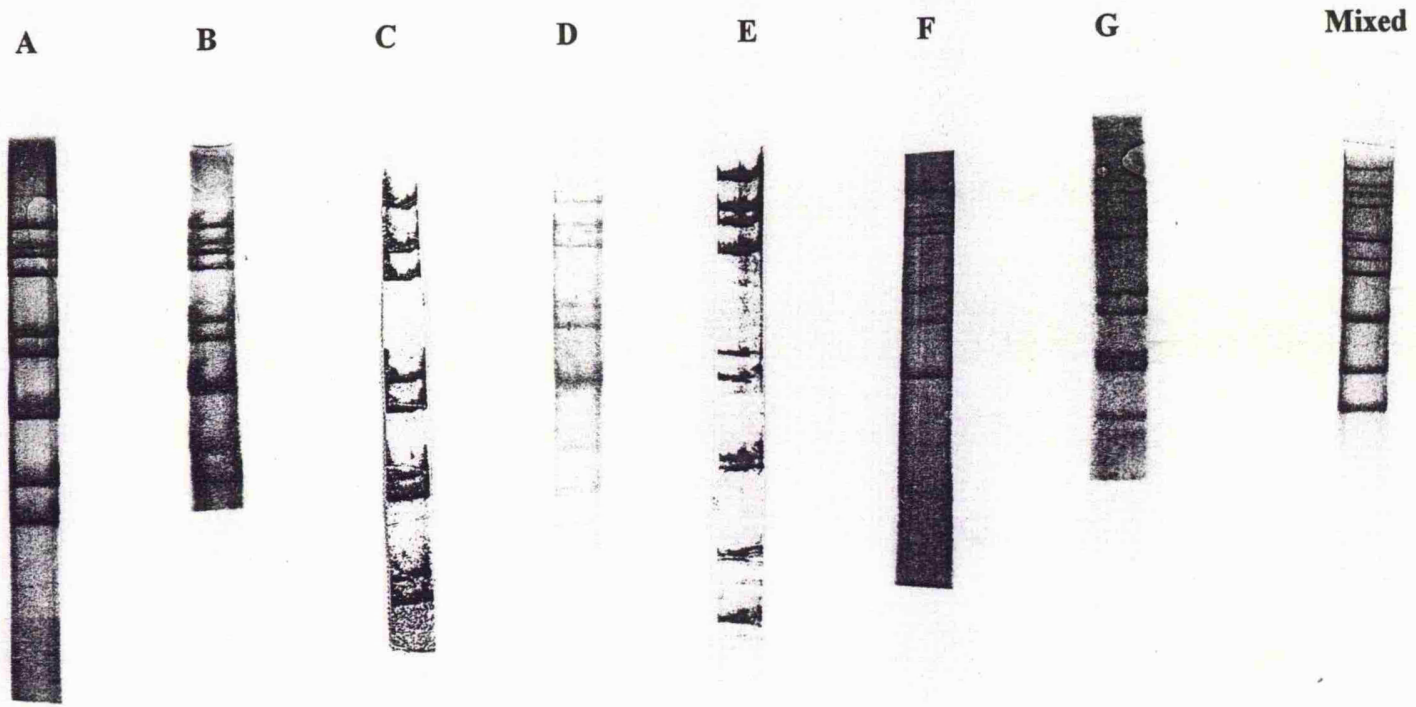


Key:

1. Groups A to G represent the seven electropherotypes

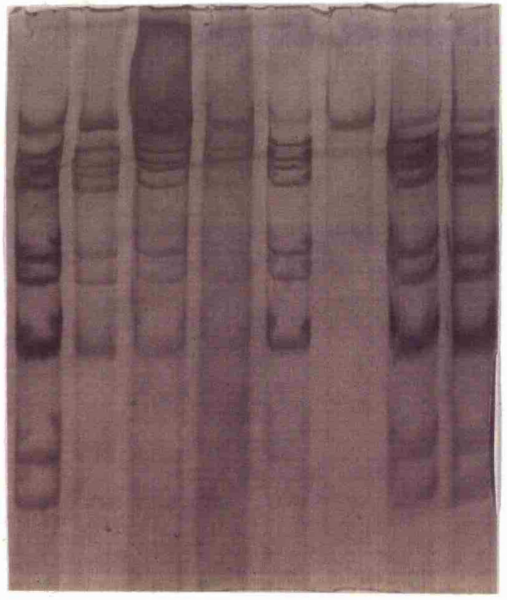
2. Groups B and G represent the short electropherotype patterns

Fig. 4.2.2. Electropherotypes of Rotavirus in patients at KFSH & RC.

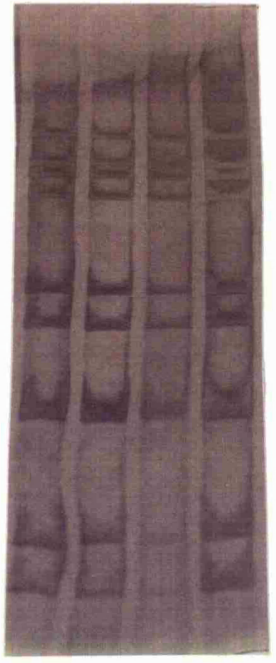


Photograhic gels were not available and so
gels were wrapped in plastic film and photocopied.

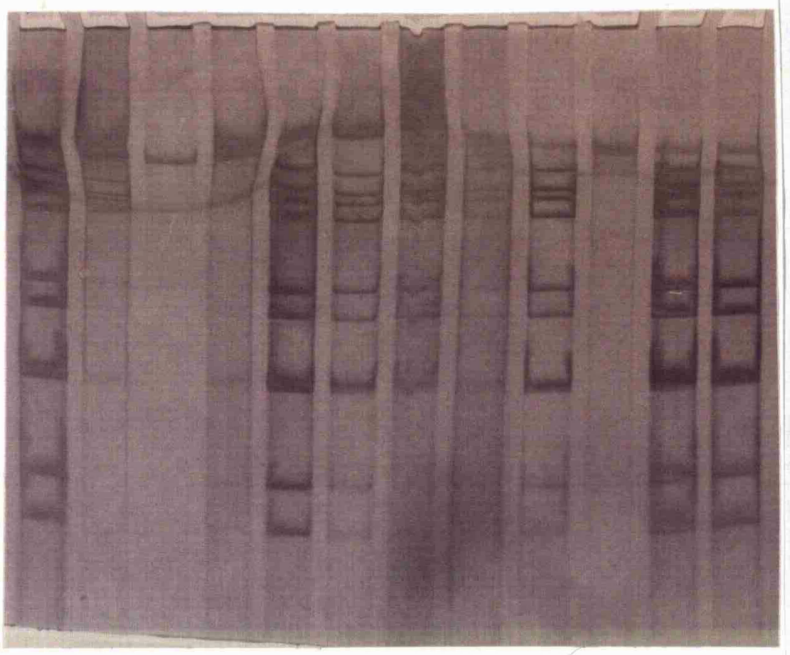
Fig. 4.2.3. Electropherotypes of Rotavirus at KFSH & RC (Modified Method).



Continous gel.



Increased current(200mA)



1mm thick gel

Discussion.

Rotavirus has a global distribution and affects mainly infants aged 6-24 months, with a peak incidence at 9-12 months. The prevalence has been determined mainly from hospital based studies which have shown rotavirus in up to 50% of patients with acute enteritis (Birch 1977). Rotavirus is most prevalent during the winter months in temperate regions but seasonal variation is less evident in tropical regions.

Gastroenteritis among children in the Middle East is common . Dutta et al. (Dutta et al., 1990) reported that in children under 5 years in Bahrain, rotavirus was the most common enteropathogen, representing 69% of the total positives. It was detected most frequently in the age group 6-11 months. A prevalence rate of up to 46% has been found in Saudi Arabia (El Assouli 1992). In this study the prevalence rate was 12% and 75% of rotavirus infections were in children 5 years or less. There was an indication of seasonal variation with more infections in the cooler months.

Epidemiology of electrophoretic patterns of segmented viral RNA by polyacrylamide gel electrophoresis is another useful tool in the epidemiology of rotavirus infections. The pattern and range of electropherotypes has differed greatly throughout the world. Studies in Bangladesh have shown 11 migration patterns (Tabassum, 1994) whereas 108 electropherotypes have been observed in England (Noel, 1991). Of the rotavirus positive samples 5 had a long

electrophoretic pattern characteristic of human rotavirus subgroup 2 and only two short patterns were observed. These results are similar to a study in Tbilisi (Ginevskaya, 1991) where 7 electropherotypes were also noted over an 18 month period.

Mohammed et al. (Mohammed et al., 1994) have carried out subgrouping and serotyping of rotaviruses in Saudi Arabia. They found that subgroup II was predominant and represented 61.3% of cases subgrouped. Subgroup I comprised 26.3% of cases. All the subgroup I were of serotype 2 and all of subgroup II belonged to serotypes 1 (54.7%) and 4 (9.4%). The remainder were untypeable. Serotyping of 355 rotaviruses revealed a distribution profile of serotype 1, 53.5%; serotype 2, 6.8%; serotype 3, 5.9%; and serotype 4, 22.8%. The electropherotypes in this study were also mostly indicative of subgroup II and serotypes 1,3 and 4.

The high prevalence of rotavirus shows the need to combat the spread of this virus through vehicles such as water, hands, fomites and food, and especially the nosocomial transmission of the infection. Such measures will greatly help to contain this relatively resistant and ubiquitous virus. Further epidemiological studies nationwide are also required in order to develop an effective vaccine.

CHAPTER 5. CONCLUSION

On a global scale, diarrhoeal diseases are the single greatest cause of morbidity and mortality, far exceeding that from heart disease, cancer or strokes in many populous areas (Guerrant, 1983). Gastrointestinal infections encompass a wide variety of symptom complexes and recognized infectious agents. Although the global mortality has decreased due to oral rehydration therapy; some areas have a worsening diarrhoea mortality and prolonged diarrhoea is emerging as the major cause of death.

The problem of chronic and persistent diarrhoeas are important in developing countries as they are prevalent in undernourished populations where one fifth of deaths in children under five years age are associated with diarrhoea, and more than half of all diarrhoeal deaths are attributed to chronic diarrhoea (Ament and Barclay, 1982). A study of 4,000 children under five in Bangladesh (Shahid et al., 1988) showed that almost 10% had chronic diarrhoea. The peak age was two with more deaths than in children with acute diarrhoea.

Living conditions are an indicator of socioeconomic conditions; type of housing, crowding, sanitation facilities, and water sources are major determinants of environmental exposure to enteric pathogens. Saudi Arabia despite being a developing country has an affluent society compared to other developing countries. There is little crowding due to a small population, sanitation facilities are in place and most water is derived from huge

desalination plants. There is also a modern infrastructure with a high standard of living for most of the population. The main problem has been that of education which is now being undertaken with an aggressive education policy. Also, due to a large number of intermarriages there are a high level of medical complications and debilitating conditions that are not seen in developed countries. This has given rise to a large number of immunocompromised patients that are vulnerable to many infectious diseases including enteric pathogens. The local population is also introduced to new parasites due to a dynamic expatriate work force largely from the third world.

If one examines the overall aetiology of gastroenteritis it can be seen that each country has its own peculiar characteristics and prevalence of different pathogens that are related to the environment, geography and socio-economic conditions. These factors need to be taken into account when developing strategies for disease prevention in each country. For example, in South Africa the most common organisms identified were 38.5% enterotoxigenic *Escherichia coli*, 25% *Cryptosporidium parvum,* 15% *Campylobacter*, 14% enteric adenoviruses and 12% rotaviruses (Geyer et al., 1993). At Gaza which has a very similar climate and geography to Saudi Arabia but is poverty stricken; children under 5 had 18.5% *Salmonella* sp., 14.6% *Cryptosporidium parvum*, 8.3% *Campylobacter* and only 6.8% rotavirus (Sallon et al., 1994). Study of parasitic infections in Indonesia showed *Entamoeba hartmanni* 16.3%, *Endolimax. nana* 12.5%, *Entamoeba histolytica* 11% and *Giardia lamblia* 4.6% (Mangali et al., 1993). In Hong Kong the commonest pathogens are *Salmonella* (45%), followed by rotavirus (34%) and *Campylobacter* (11%) (Ling and Cheng, 1993). The results of this study showed that *Salmonella* was the most common bacterial pathogen (52%) and *Giardia lamblia* the most common parasite. Rotavirus was the commonest viral infection followed by adenoviruses. However, *Cryptosporidium parvum* was almost absent in this country which

has been corroborated in other studies (Bolbol 1992); whereas in other developing countries such as Malawi higher incidence rates (9%) have been reported (Pavone et al., 1990).

Antibiotics.

Antibiotics are generally indicated with bacterial diarrhoeas in certain circumstances. It has been suggested that antibiotics are needed in children with chronic disease or where sepsis is recognized. For example, enteric fever caused by *Salmonella typhi* can be treated with antibiotics. The availability of chloramphenicol has greatly reduced mortality in typhoid but chloramphenicol-resistance has become a problem. In severe typhoid combinations of chloramphenicol and dexamethasone are recommended. Appropriate treatment depends on the result of sensitivity tests.

Asymptomatic carriers of typhoid *Salmonella* can also be treated. Amoxycillin has been shown to cure 73% of carriers (Nolan and White, 1978). The combination of ampicillin and probenecid has achieved cure in 58% of carriers (Edelman and Levine, 1986). This compares with reports of 100% success with treatment intravenously with ampicillin for 2 weeks. With Vibrios antibiotics such as tetracycline and chloramphenicol are indicated but again the problem of resistance is starting to emerge.

The use of antibiotics is also recommended for severe infections in young infants and debilitated patients. However, since multiple resistant strains are common,

treatment must be based on sensitivity tests. The emphasis on proper testing cannot be overemphasised in developing countries where the problem is most critical. In this study the resistance patterns of 14 antimicrobial agents to the *Enterobacteriaceae* were found to increase over a six year period and can be related to overuse and mismanagement of these illnesses. Another important factor is that health services are totally free for the Saudi population including all prescriptions. Hence, this easy availability has led to a lack of regard for the limitations of these antimicrobial agents.

Specific treatments for viral gastroenteritis have not been devised. In a recent study the activity of halogeno-, cyano-, and amidino-isoflavenes, isoflavans and flavans was studied *in vitro* on the multiplication of astroviruses. All these agents caused a dose-dependent reduction of viral antigen, but the chloroderivatives were the most effective (Superti et al., 1990). The use of glycoproteins or glycolipids that compete with enterocytes for binding of rotavirus has been proposed as passive therapy or for transient prevention (Willoughby et al., 1990).

Viruses.

There has been significant progress in defining the aetiology of viral enteritis. Rotavirus is the major cause of diarrhoea in children in both developed and developing countries. Adenoviruses have also emerged as a major cause of diarrhoea with an increasing number of serotypes being implicated. This study showed as in other parts of the world that adenoviruses are the second most

common cause of viral gastroenteritis. Types 40/41 were most prevalent and appeared mostly between August to December. The other adenoviruses showed no seasonal variation. Adenoviruses are important enteric pathogens in the immunocompromised host but it is not always fully realized that the immunocompetent patient can also be infected by non-enteric serotypes of adenovirus (Krajden et al., 1990). Adenoviruses are not generally diagnosed by physicians despite their serious implication in immunocompromised patients at this institute. This may be partly due to no treatment being available. No reports of gastrointestinal adenoviruses or serotyping have been carried out to date in Saudi Arabia. It is hoped that this situation will rapidly change with the availability of simple and rapid tests for their identification. However, serotyping will be more difficult since specialized facilities are required and can only be carried out at larger centres such as this one.

If one combines the pool of bacteriological and virological data, an aetiological agent is undetected in 20-30% of cases. Therefore, there is still much to learn of the pathogenesis of some emerging small round viruses such as astroviruses, caliciviruses, Norwalk etc.. Epidemiological studies on SRSVs have been limited by the problems of establishing them in cell culture and inability to transmit them to animals. The use of EM and development of new techniques such as RIA and EIA have shown that they are a cause of outbreaks and sporadic cases of diarrhoea and vomiting. Evidence has also shown that polluted water, molluscan shellfish, and contaminated cold food are major sources of infection. Improved methods of detection are required to contain these devastating diarrhoeal diseases. At this

centre the use of PCR gave a relatively low incidence of astrovirus (1.5%). But this situation will undoubtedly change as more centres in this country start to examine for these viral agents and determine which serotypes are present. A study in Oxford, England has shown evidence for new serotypes of astrovirus (Lee and Kurtz, 1994) and this may also prove to be the case in this country. Another case for nationwide testing is that the climate and patient characteristics can change from location to location in this large country roughly the size of Europe.

Vaccines.

Vaccines have been responsible for eradicating many diseases in the developed countries. A prime target for disease prevention in the developing world has been diarrhoeal diseases since they are a major cause of morbidity and mortality. However, vaccine development is a complicated process involving a thorough understanding of epidemiology, pathogenesis, molecular biology and vaccine delivery.

There has been no new human diarrhoeal vaccine licensed for use in over a decade. Among bacteria, there has been some progress in the development of vacccines. These include an oral killed B-WC vaccine and a killed WC-alone vaccine against cholera, and both a live attenuated oral vaccine and an injectable Vi antigen vaccine against typhoid fever. In addition, a killed oral ETEC vaccine and live attenuated oral *Shigella* vaccines have begun to be tested in humans. These vaccines hold promise in control programmes in developing countries (Holmgren

and Svennerholm, 1992). There has been little progress for vaccines against protozoan causes of diarrhoea.

Of the many viral causes of infectious diarrhoea, potential vaccines are currently only being studied for rotavirus. A quadrivalent rotavirus vaccine consisting of rhesus rotavirus vaccine and three reassortment viruses is undergoing placebo-controlled field trials in the U.S.A. and in two developing countries. Experimental vaccines against enteric adenovirus infections have not yet been developed. Major obstacles are their fastidious growth requirement since they only grow in transformed cell lines. Although not oncogenic they have been shown to transform rat embryo cells. Live oral vaccines against adenovirus types 4 and 7 have been successfully used to treat military recruits. However, these are administered in enteric coated capsules since the principle is based on the fact that types 4 and 7 replicate in the gut but do not induce symptoms. This would not be applicable to the enteric adenoviruses.

Diagnosis.

A specific diagnosis is important since unecessary antibiotic treatment can be avoided, additional expensive enteric bacterial cultures or examination for parasites may not be needed. Also, spread of virus to other patients and staff can be prevented by infection control measures.

The cost of a stool examination on every patient for all potential pathogens is prohibitive and even more so in developing countries. Therefore, strategies

should be developed to ensure the highest possible positivity rate. Factors to be considered include the age and hospitalization status of the patient, season, geographic locale, and histories of recent travel, diet, and underlying disease.

Due to additional constraints in developing countries several methods in this study were evaluated for their efficacy. Latex methods proved to be useful screening methods where laboratory support is absent but in larger facilities more sophisticated methods such as EIA , EM and PCR are necessary for greater specificity and sensitivity. Lack of finances and resources are often the problem in developing countries and simple methods are often needed. Mizanur-Rahman (Mizanur-Rahman, 1990) has shown that despite a modest sensitivity, the presence of neutral fats in stool may be a good indicator for identifying rotavirus diarrhoea especially because it is simple and cost effective.

Most cases of outpatient diarrhoea in adults are inflammatory diarrhoeas, and stools can be screened for leukocytes with a methylene blue stain. The sensitivity is less than 90% but but it can help to eliminate agents of non-inflammatory diarrhoea such as toxins produced by vibrios and ETEC, viral agents, and certain parasitic agents. In the case of nosocomial infections, viruses such as rotavirus and enteric adenoviruses should be primarily considered in children as the leading cause of nosocomial diarrhoea.

Advances in technology are rapidly bringing changes to laboratory practices, especially with the impact of nucleic acid hybridization and gene amplification

techniques. A number of bacterial, viral and parasitic enteric pathogens have been identified with DNA or RNA probes. New kits have already been described for rotavirus that can detect both group A and non A with increased sensitivity of detection over conventional immunoassays (Kinney et al., 1989).

Future Work.

There has been little development in the area of environmental epidemiology for monitoring food and water supplies as reservoirs of infection in Saudi Arabia. This is probably an important factor due to the hot conditions for food spoilage that exist in this country and the very long coastline. Samples of water have been collected from different sources throughout Saudi Arabia including sea water, well water, reservoirs, swimming pools and rivers. Samples were ultra-centrifuged, desiccated and frozen away for viral analysis. It is hoped to apply PCR techniques to these samples when more readily available.

This study has made an attempt to give an insight on the nature of gastrointestinal diseases in Saudi Arabia with particular emphasis to viral infections. However, the patient population examined in this population was relatively specialized and it will be necessary to examine the general Saudi population in order to assess the true significance of many pathogens in the community. It would also be interesting to continue this study over a longer period. This would allow for more detailed analysis on any seasonal variations and particular serotypes that re-emerge. The

information could also be related to the level of sanitation, disinfection, food hygiene and personal hygiene such as hand washing. It is hoped to carry out a follow-up survey on environmental contamination of rotavirus, after a programme of disinfection has been initiated, to show whether environmental contamination actually decreases.

The emphasis was on viral aetiologies since these have been little studied due to the lack of appropriate facilities and expertise. The serotypes of adenoviruses most prevalent in this country have now been characterized providing knowledge on the epidemiology of the disease. There has been very little information on other viral causes of gastroenteritis such as SRSVs and it is important to introduce simple and sensitive new procedures for their detection such as PCR. New technologies will also allow for detection of emerging gastrointestinal viruses such as pestiviruses, picobirnaviruses and parvoviruses. However, these are still research tools and improvements in diagnostics will only come with the introduction of standardized commercial kits and automation.

Appendix 1 - Tables of antimicrobial susceptibility patterns of clinical isolates at KFSH & RC

Table 1. Antimicrobial Susceptibility of Clinical Isolates at KFSH & RC during 1988-1994

Organism (n)	AM	AN	AUG	CF	CAZ	C.FOX	FUR	GM	NET	PIP	SXT	TE	IMP **	CIP **
E. COLI														
1988/89 (832)	40	95	68	85	100	92	96	92	93	51	55	33	100	--
1990 (1766)	38	95	62	54	98	92	93	88	92	46	56	40	100	88
1991 (1810)	39	94	54	53	97	95	92	90	93	45	55	34	100	99
1992 (1549)	37	--	54	71	96	96	92	91	94	41	54	38	100	93
1993 to Mar 94 (1635)	28	93	57	70	94	97	--	92	69	33	47	29	100	94
MORGANELLA														
1988/89 (42)	10	100	5	10	81	60	0	81	90	62	57	29	100	--
1990 (81)	2	94	4	0	89	15	12	90	99	80	63	42	--	--
1991 (64)	6	95	6	3	95	13	0	91	97	88	73	38	100	100
1992 (69)	0	96	0	0	93	19	23	83	96	72	82	46	100	100
1993 (40)	3	98	8	8	93	25	0	96	100	76	63	36	100	93
KLEBSIELLA SP.														
1988/89 (92)	13	100	69	69	100	91	50	94	94	81	81	69	100	--
1990 (71)	1	92	77	62	100	92	70	69	82	62	66	69	100	--
1991 (66)	8	92	77	60	97	96	67	87	94	76	89	71	100	--
1992 (72)	11	73	57	49	78	87	84	75	92	62	68	62	100	--
1993 (86)	0	60	68	44	73	89	--	61	13	44	60	50	100	--
K. PNEUMONIAE														
1988/89 (276)	1	90	72	80	97	97	69	81	88	70	73	51	100	--
1990 (693)	3	88	70	72	92	88	51	77	83	66	71	58	99	100
1991 (787)	1	77	65	70	84	91	34	75	75	61	68	28	100	91
1992 (656)	1	69	64	70	76	91	40	73	69	48	69	34	100	95
1993 (770)	1	82	68	76	82	95	--	83	53	42	73	28	95	94

PROTEUS SP.	AM	AN	AUG	CF	CAZ	C.FOX	FUR	GM	NET	PIP	SXT	TE	IMP **	CIP **
1988/89 (126)	40	95	68	85	100	92	96	92	93	51	55	33	100	--
1990 (206)	66	97	88	74	100	77	97	90	97	88	75	8	--	--
1991 (225)	57	100	79	65	98	92	0	88	98	88	72	7	84	94
1992 (213)	60	97	85	69	99	90	0	88	95	84	71	10	88	99
1993 (226)	58	98	89	73	99	96	--	88	78	82	62	2	89	100
CITROBACTER														
1988/89 (32)	6	94	33	63	94	75	100	--	94	81	75	63	100	--
1990 (63)	6	84	11	13	62	11	92	--	75	41	59	46	95	73
1991 (70)	9	89	26	25	79	29	82	--	83	63	70	50	100	100
1992 (66)	0	96	30	26	61	32	94	--	45	52	68	55	100	72
1993 (91)	3	98	8	8	93	25	0	96	100	76	63	36	100	93
ENTEROBACTER SP.														
1988/89 (162)	13	88	16	47	84	24	67	81	88	72	66	31	100	--
1990 (242)	2	87	3	2	78	3	53	82	83	70	75	44	100	100
1991 (168))	7	82	13	15	75	15	48	82	83	67	86	32	100	95
1992 (105)	4	83	19	18	64	34	46	83	79	56	87	36	100	99
1993 (126)	0	88	27	32	94	34	--	87	88	52	87	28	100	94
S. MARCESCENS														
1988/89 (66)	0	74	0	0	100	13	0	43	65	39	57	0	100	--
1990 (134)	1	74	1	0	100	3	0	46	72	51	57	1	100	96
1991 (139)	2	52	2	0	97	22	0	44	48	53	70	0	100	100
1992 (100)	12	56	10	0	73	30	6	66	66	60	82	2	100	100
1993 (161)	6	53	6	0	57	23	--	70	27	53	80	0	100	91

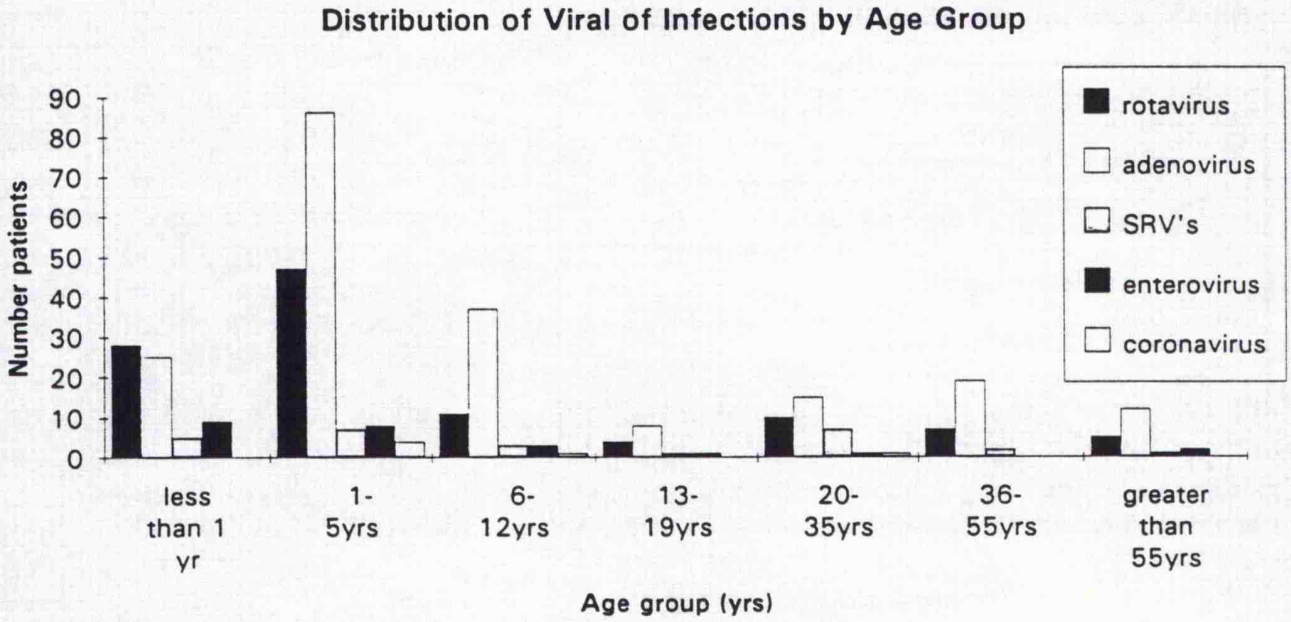
SALMONELLA SP.	AM	AN	AUG	CF	CAZ	C.FOX	FUR	GM	NET	PIP	SXT	TE	IMP **	CIP **
1988/89 (60)	85	100	90	95	100	--	50	100	100	100	75	55	100	--
1990 (196)	84	--	90	99	--	--	--	98	--	--	90	61	--	--
1991 (349)	72	100	77	85	95	--	--	96	--	--	89	45	--	--
1992 (202)	71	--	71	--	99	--	--	--		--	81	--	--	--
1993 (253)	82	--	82	--	96	98	--	--	--	--	74	--	--	--
SHIGELLA SP.														
1988/89 (28)	50	100	75	50	--	--	--	75	--	--	--	--	100	--
1990 (78)	49	--	66	85	--	--	--	100	--	--	22	21	--	--
1991 (66)	59	93	76	87	100	--	--	99	--	--	21	24	--	--
1992 (27)	47	--	--	--	100	--	--	--	--	--	16	13	--	--
1993 (30)	69	--	--	--	100	--	--	--	--	--	13	20	--	--

AM= ampicillin; AUG= augmentin; PIP= piperacillin; CF= cephazolin; CFOX= cefoxitin; CAZ= ceftazidime; AN= amikacin; GM= gentamicin; NET= netilmycin; FUR= nitrofurantoin; TE= tetracycline; TMP-SX= trimethoprim-sulfamethoxazole; IMP= imipenem; CIP= ciprofloxacin

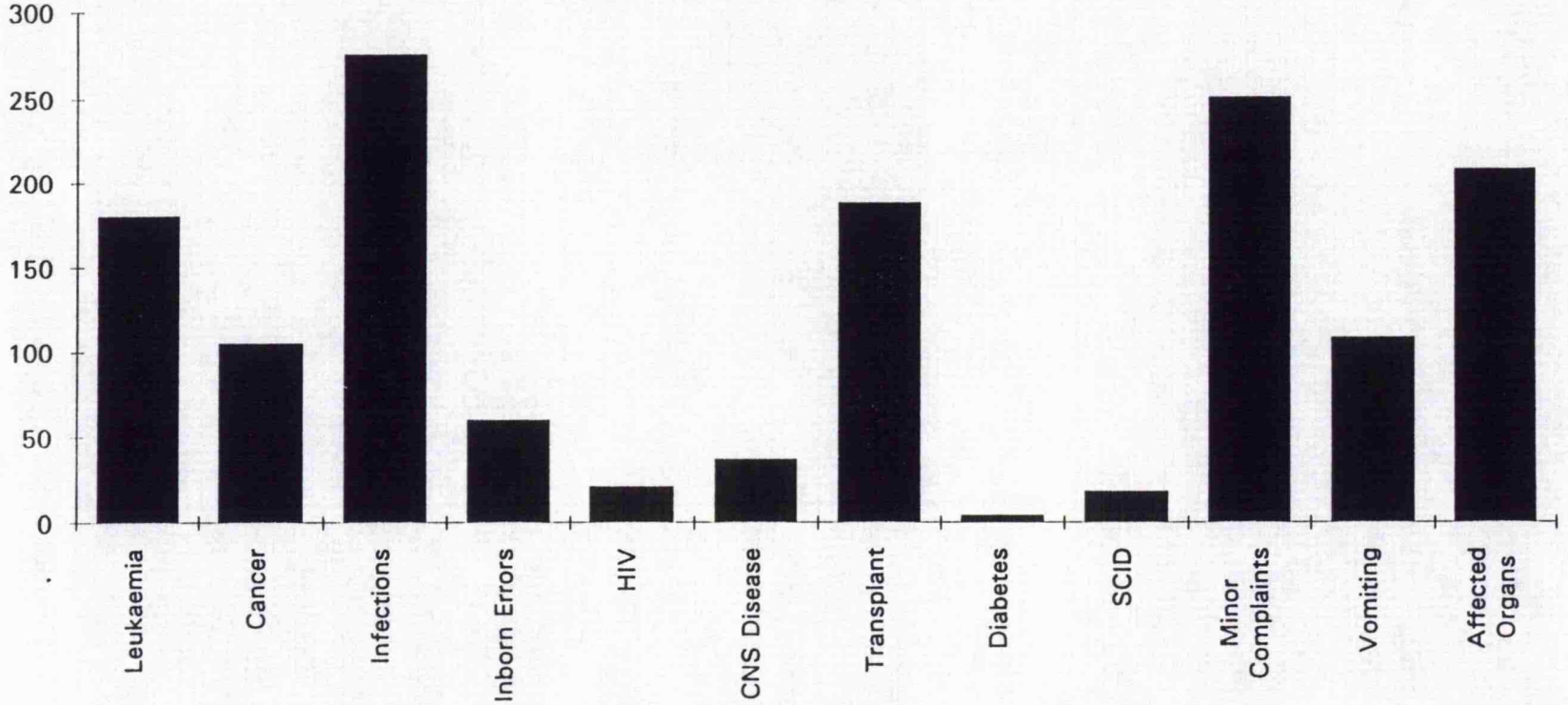
n = number of isolates tested

** *Tested on multiresistant strains only*

Appendix 2 - Data analysis of 3000 diarrhoeal specimens at KFSH & RC

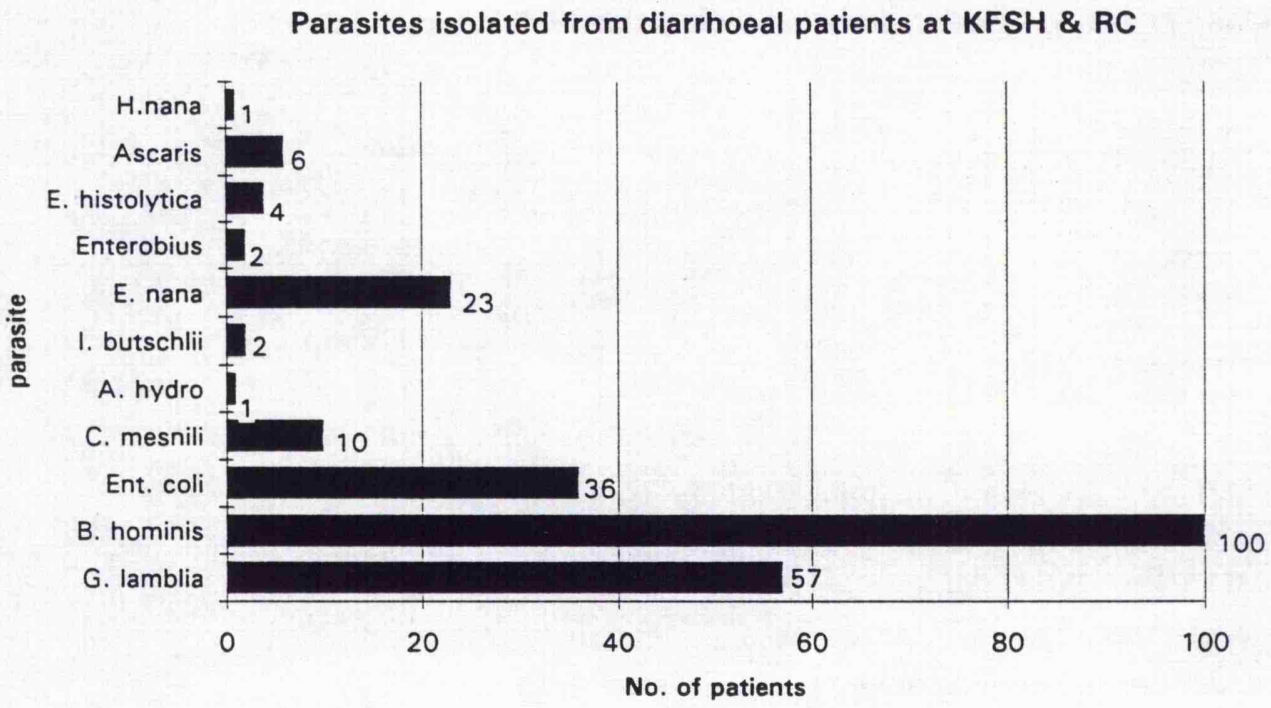

Distribution of Viral of Infections by Age Group
Number patients
90
80
70
60
50
40
30
20
10
0
rotavirus
adenovirus
SRV's
enterovirus
coronavirus
less than 1 yr
1-5yrs
6-12yrs
13-19yrs
20-35yrs
36-55yrs
greater than 55yrs
Age group (yrs)

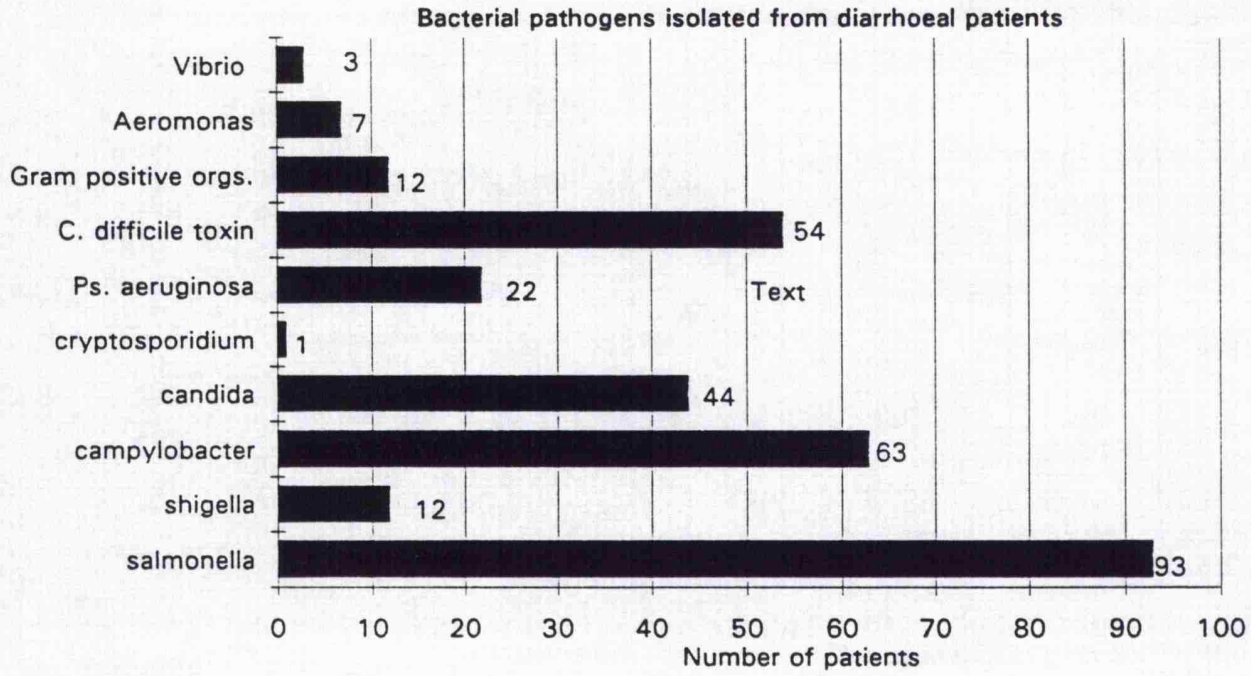
Clinical Diagnosis of Patients with Gastrointestinal Adenovirus Infections.

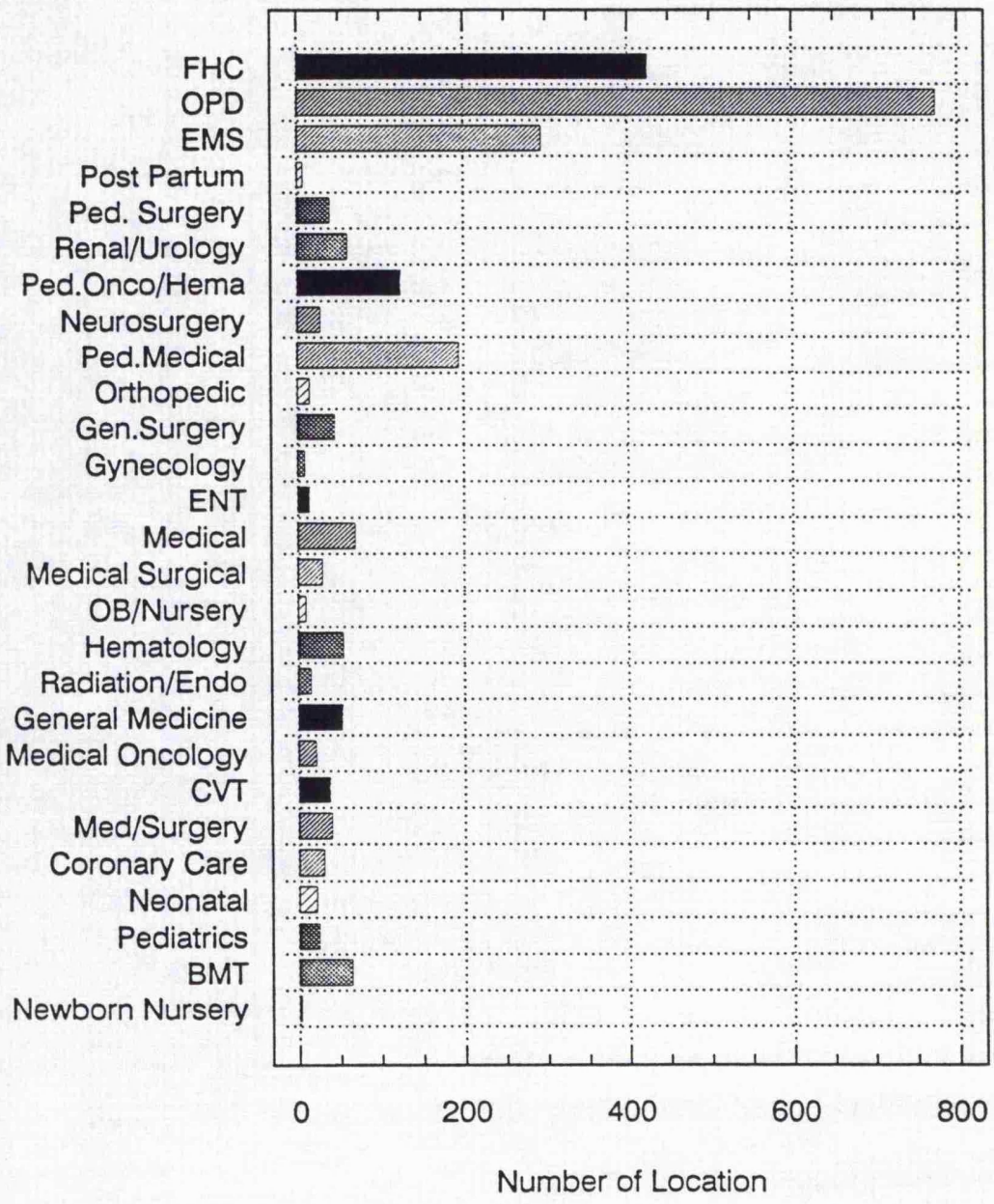


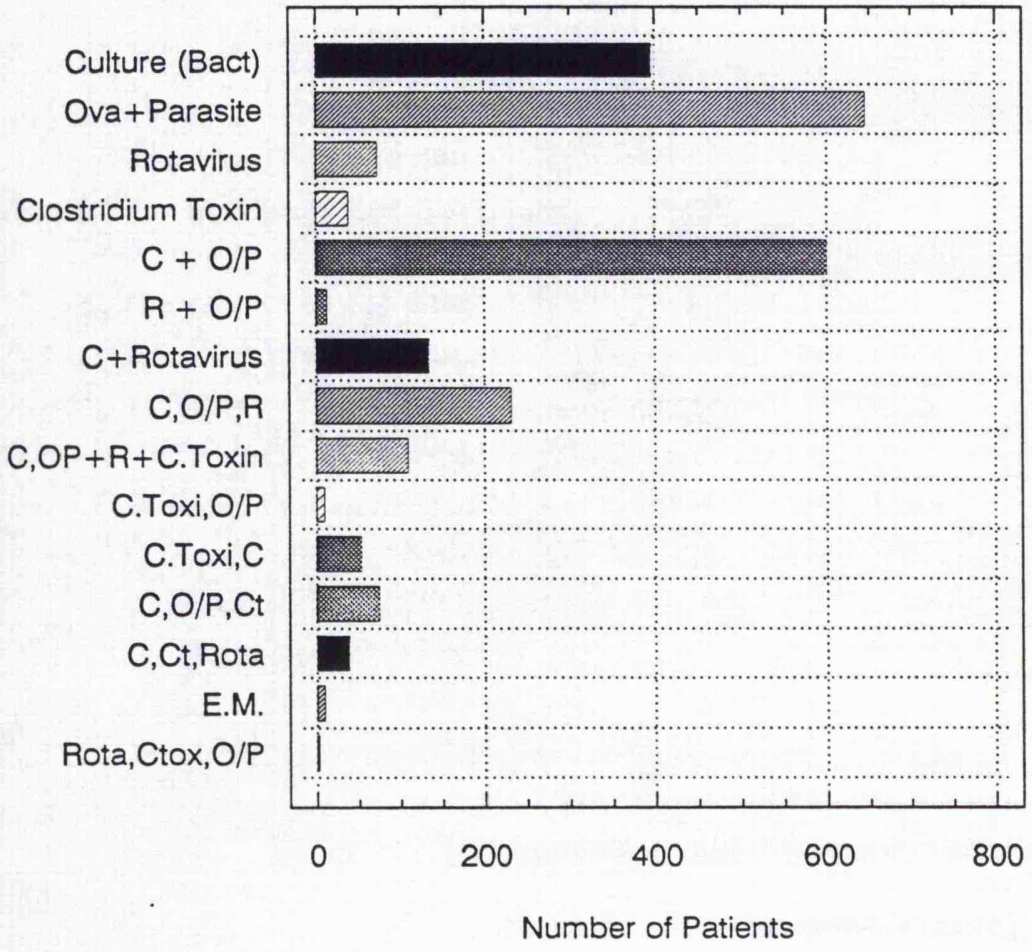
Distribution of Adenovirus Serotypes in Patients at KFSH & RC.

	Adenovirus serotype																						
Date	No type	40/41	1	2	3	4	5	6	7	8	9	10	8,10	12	13	15	17	19	20	22	24	26	27	28
Jun-92	1			1	2		2																1	
Jul-92														1										
Aug-9	1	3																						
Sep-92		1	1													1								
Oct-92																								
Nov-92	6	6	1				1		1															
Dec-92	3	6	4	1			1										1				1			
Jan-93	1	3		1							1													1
Feb-93	2	1	3	4	1		1	1		1												1		
Mar-9	3	1	4		4					1				1										
Apr-93	2	2	1	1																	1			
May-93		1					1																	
Jun-93																								
Jul-93		3		1	1	2	3	1	2		1					2			1					
Aug-93	4	9	1	1	1		2	1		1		2		1										
Sep-93	7	2	3		3	4	2			1	1													
Oct-93	1	1							1							1								
Nov-93		1						1			1													
Dec-9	1	1					1	1									1							
Jan-94	1	3		2			4																1	
Feb-94							1			1														
Jun-94		2		4				1						1				1		1				
Jul-94		3	1	4			1		1														1	
Aug-94		5		2				1					1			1							1	
Sep-94					1								1		1									
Oct-94	1	3	1																					
Total	34	57	20	22	13	6	20	7	5	5	4	2	2	4	1	5	2	1	1	1	2	1	4	1

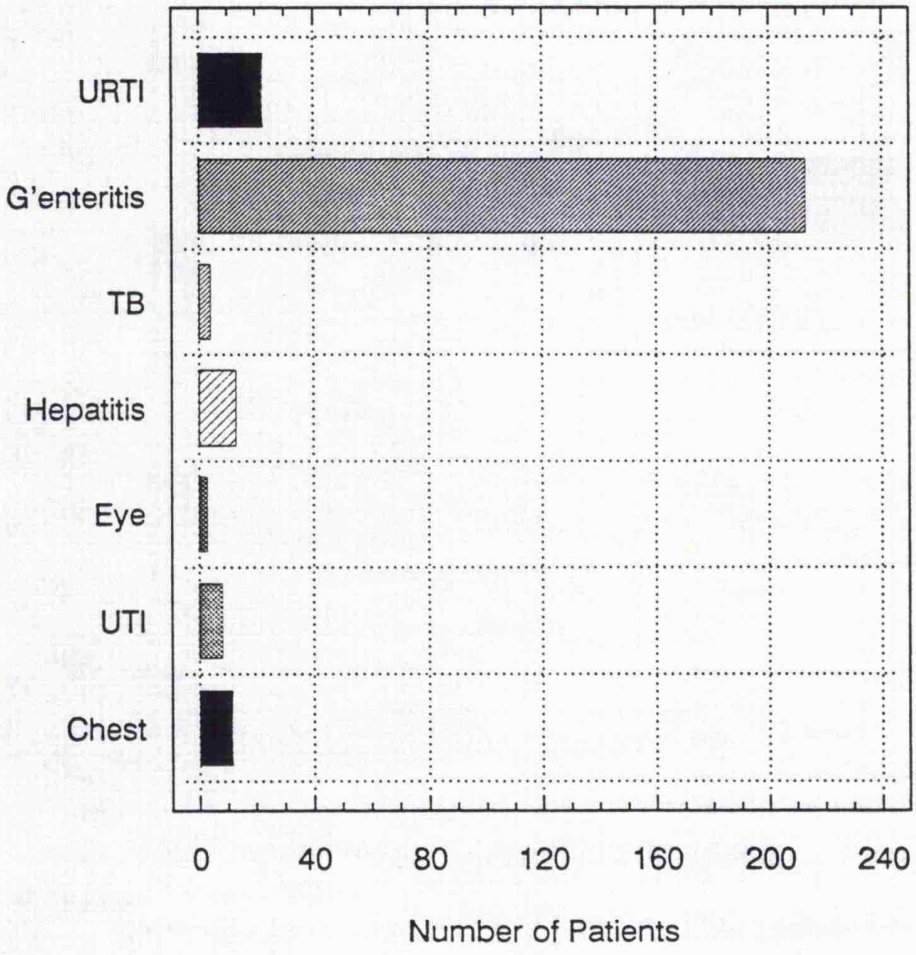

Parasites isolated from diarrhoeal patients at KFSH & RC
parasite
H.nana
Ascaris
E. histolytica
Enterobius
E. nana
I. butschlii
A. hydro
C. mesnili
Ent. coli
B. hominis
G. lamblia
1
6
4
2
23
2
1
10
36
100
57
0
20
40
60
80
100
No. of patients

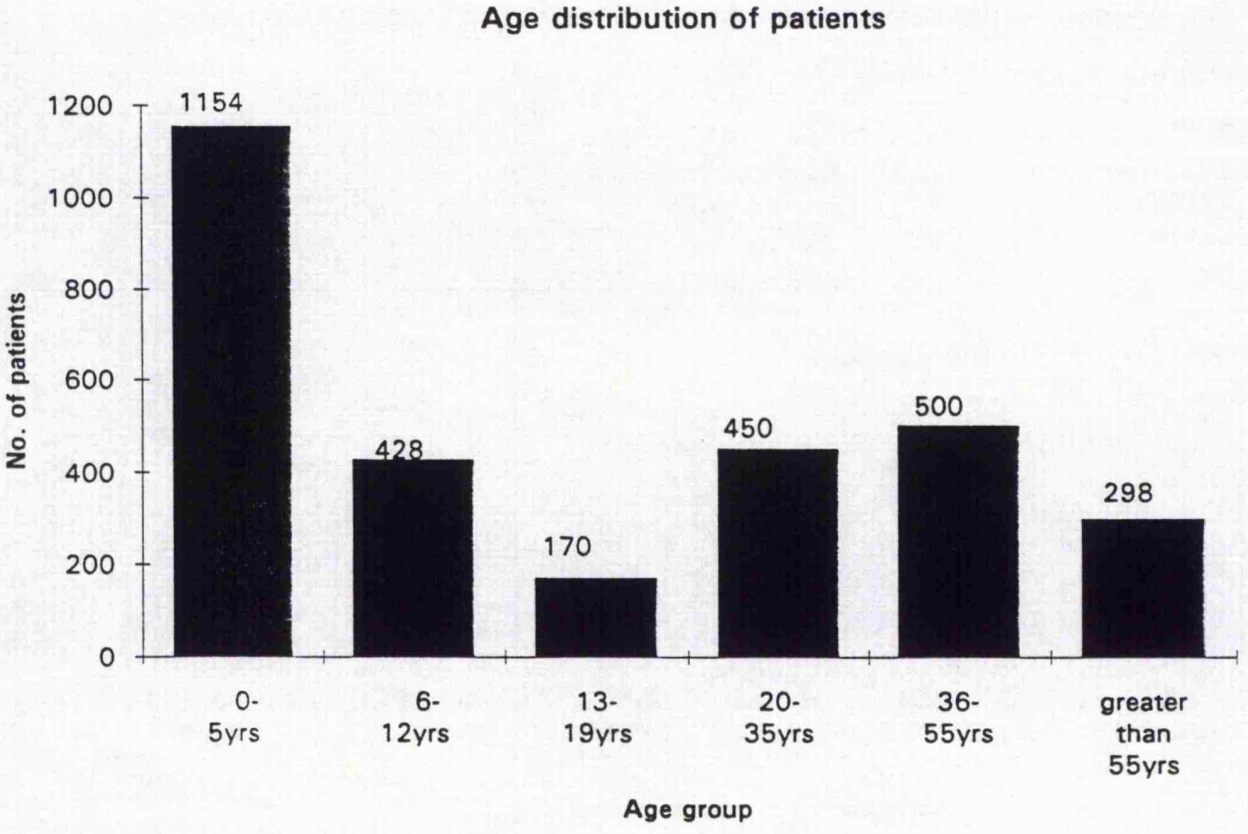

Bacterial pathogens isolated from diarrhoeal patients
Vibrio
3
Aeromonas
7
Gram positive orgs.
12
C. difficile toxin
54
Ps. aeruginosa
22
Text
cryptosporidium
1
candida
44
campylobacter
63
shigella
12
salmonella
93
0
10
20
30
40
50
60
70
80
90
100
Number of patients


WARD LOCATION
FHC
OPD
EMS
Post Partum
Ped. Surgery
Renal/Urology
Ped.Onco/Hema
Neurosurgery
Ped.Medical
Orthopedic
Gen.Surgery
Gynecology
ENT
Medical
Medical Surgical
OB/Nursery
Hematology
Radiation/Endo
General Medicine
Medical Oncology
CVT
Med/Surgery
Coronary Care
Neonatal
Pediatrics
BMT
Newborn Nursery
0
200
400
600
800
Number of Location

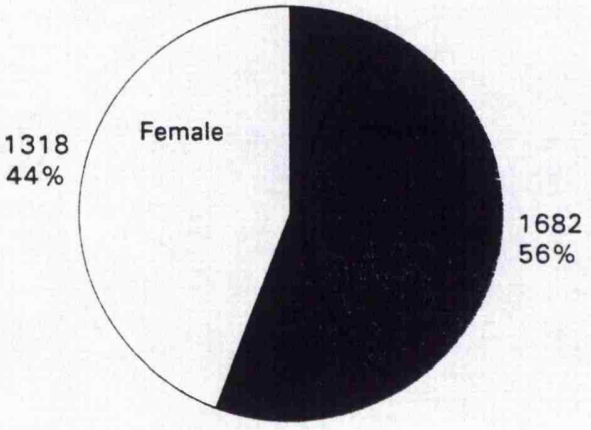

TYPE OF LABORATORY TEST
Culture (Bact)
Ova+Parasite
Rotavirus
Clostridium Toxin
C + O/P
R + O/P
C+Rotavirus
C,O/P,R
C,OP+R+C.Toxin
C.Toxi,O/P
C.Toxi,C
C,O/P,Ct
C,Ct,Rota
E.M.
Rota,Ctox,O/P
0
200
400
600
800
Number of Patients

INFECTIONS PRESENT IN PATIENT POPULATION




Age distribution of patients
No. of patients
1200
1000
800
600
400
200
0
1154
428
170
450
500
298
0-
5yrs
6-
12yrs
13-
19yrs
20-
35yrs
36-
55yrs
greater
than
55yrs
Age group

Ratio of Male to Female Patients



Appendix 3 - Bacteriological media, methods and controls

3.1. Selective media for the isolation of the *Enterobacteriaceae*.

Medium	*Components*	*Colonial morphology*	*Uses*
MacConkey's agar	Selective agent: bile salts. Indicator system: lactose plus neutral red pH indicator.	Lactose fermenters red or dark pink; late lactose fermenters pale pink. Non-lactose fermenters colourless or yellow (due to alkaline amine production from protein). Faecal streptococci tiny, magenta colonies.	Low selectivity for salmonellae and shigellae. Can be used for the isolation of *E.coli* and *Yersinia enterocolitica*
Hektoen enteric agar (HEA)	Selective agent: bile salts. Indicator systems: three sugars (lactose, sucrose, salicin); pH indicator is thymol blue (yellow in acid conditions). A dye, acid fuschin, is included. As with DCA and XLD, there is a detection system for H_2S.	Salmonellae and shigellae do not ferment any of the sugars (apart from rare strains) and give green or blue-green colonies (the colour of the uninoculated medium). Organisms fermenting one or more of the sugars give yellow or orange-yellow colonies (the orange tint is due to the dye). H_2S producers give black-centred colonies.	General plating medium but more selective than XLD. Useful for the isolation of salmonellae.
Campylobacter medium	Blood agar base made selective with three antibiotics, vancomycin, polymyxin B and trimethoprim. Selectivity is increased by incubating at 43°C.	Most other bacteria will not grow on this medium. *Campylobacter jejuni/coli* produces large, flat, glistening grey colonies; often, the long axis of the colony lies along the inoculation streak.	A selective medium for *Campylobacter jejuni/coli*

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32. Biochemical reactions of some enteric Gram-negative bacilli.

	Escherichia coli	*Klebsiella*	*Citrobacter*	*Enterobacter*	*Yersinia enterocolitica*	*Proteus mirabilis*	*Proteus vulgaris*	*Morganella morganii*	*Salmonella typhi*	*Salmonella* spp.	*Shigella dysenteriae*	*Shigella flexneri*	*Shigella boydii*	*Shigella sonnei*	*Vibrio cholerae*	*Vibrio parahaemolyticus*
Motility	+	−	+	+	−	−	+	−	+	+	−	−	−	−	+	+
VP reaction	−	+	−	+	−	v	−	−	−	−	−	−	−	−	v	−
Citrate utilization	−	+	+	+	−	v	v	−	−	+	−	−	−	−	−	+
Urease	−	+	−	−	+	−	+	−	−	−	−	−	−	−	−	−
H_2S	−	−	v	−	−	+	+	−	+	+	−	−	−	−	−	−
Indole	+	−	v	−	v	−	−	−	−	−	v	v	v	−	−	+
LDC	+	v	−	v	−	−	−	−	+	+	−	−	−	−	−	+
ODC	v	−	v	+	+	+	−	−	−	+	−	−	−	+	+	+
PPA	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
ONPG	+	+	+	+	+	−	−	−	−	−	v	−	−	+	+	−
Gas from glucose	+	+	+	+	−	+	+	+	−	+	−	−	−	−	−	−
Acid from:																
mannitol	+	+	+	+	+	−	−	−	+	+	−	+	−	−	−	+
sucrose	v	+	v	+	+	−	+	−	−	−	−	−	−	−	−	−
salicin	v	+	v	+	−	−	−	−	−	−	−	−	−	−	−	−
dulcitol	v	v	v	v	−	−	−	−	−	+	−	−	−	−	−	−

Key: + = most strains positive; − = most strains negative; v = some strains positive, some negative.

Screening biochemical tests for salmonellae and shigellae.

Test	*Media*	*Positive result*
O-Nitrophenyl galactosidase (ONPG) activity	Tube of *O*-nitrophenyl galactose broth.	Colour change from colourless to yellow.
Urease activity	Urea slope: peptone agar plus urea and phenol red.	Colour change from pale yellow to bright pink.
Motility	Tube of peptone water: after 4–6 h incubation look for motility in a wet preparation.	Motile organisms seen. be careful to distinguish this from Brownian motion. motile organisms move purposefully and in different directions.
Indole production	Tube of peptone water (use the tube set up for motility): after incubation, add Kovac's reagent	Red ring produced on top of the peptone water within the Kovac's reagent.
Hydrogen sulphide production	(1) Tube of peptone water. Insert lead acetate paper between cap and tube.	Blackening of the paper due to the formation of lead sulphide.
	(2) Triple sugar iron agar.	Blackening of the medium due to formation of ferric sulphide.
Carbohydrate fermentation	Sugar media—peptone water plus fermentable carbohydrate plus pH indicator.	Colour change due to acid production (dependent on indicator used).

Note: unless otherwise stated, all tests are incubated for 24 h at 37°C.

Reactions in Kligler's iron agar and triple sugar iron agar.

Butt	*Slope*	*H_2S*	*Interpretation*
Acid	Acid	No	*E.coli*, *Klebsiella* spp., *Enterobacter* spp., some *Citrobacter* strains
Acid	Acid	Yes	Some *Citrobacter* strains
Acid	Alkaline	No	*Shigella* spp., some *Proteeae*, occasional *Citrobacter* strains
Acid	Alkaline	Yes	*Salmonella* spp., *Proteeae*, some *Citrobacter* strains
Alkaline	Alkaline	No	Non-fermenting bacteria, e.g. *Pseudomonas* spp.

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3.3. Sensitivity of Control Strains

Antimicrobial	Disc Potency	Zone diameter of inhibition (mm)	
		E. coli (ATCC 25922)	*P. aeruginosa* (ATCC 27853)
Amikacin	10 μg	18–24	15–22
Ampicillin	10 μg	15–20	—
Bacitracin	10 U	—	—
Carbenicillin	100 μg	24–29	20–24
Cephalothin	30 μg	18–23	—
Chloramphenicol	30 μg	21–27	6
Clindamycin	2 μg	—	—
Colistin	10 μg	11–15	12–16
Erythromycin	15 μg	8–14	—
Gentamicin	10 μg	19–26	16–21
Kanamycin	30 μg	17–25	6
Methicillin	5 μg	—	—
Nalidixic acid	30 μg	21–25	—
Neomycin	30 μg	17–23	—
Nitrofurantoin	300 μg	20–24	—
Penicillin G	10 U	—	—
Polymyxin B	300 U	12–16	—
Streptomycin	10 μg	12–20	—
Sulfamethoxazole-trimethoprim	25 μg	24–32	—
Sulfonamide[1]	300 μg	22–26	6
Tetracycline	30 μg	18–25	9–14
Tobramycin	10 μg	18–26	19–25
Vancomycin	30 μg	—	—

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Appendix 4 -Astrovirus PCR from samples in Saudi Arabia and the United Kingdom.

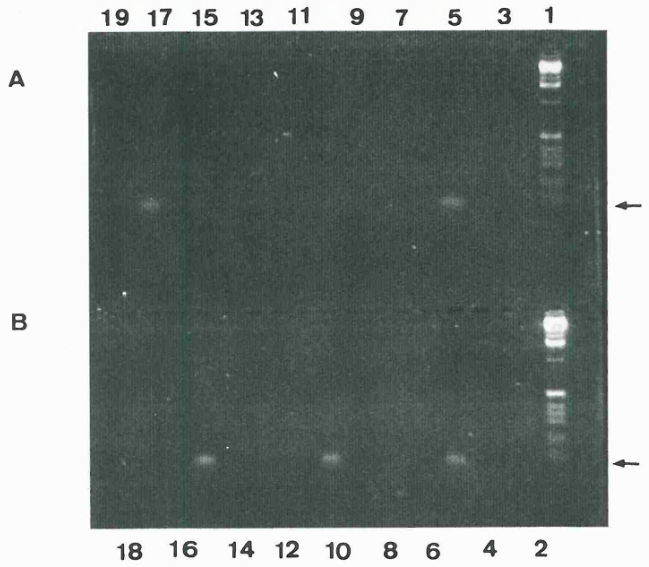

Fig. 2. RT-PCR of Leicester isolates. Five of the astrovirus positives from Leicester, UK are shown (lanes 5 and 18 in A, lanes 5, 10 and 15 in B).

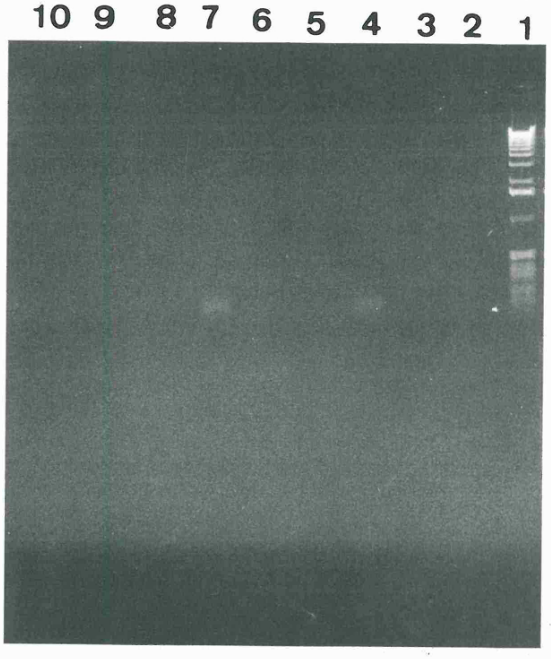

Fig. 1. RT-PCR of Saudi samples. Astrovirus PCR positives from Saudi Arabia (lanes 4 and 7). The arrow indicates the positive bands of 110 bp. The molecular weight marker is a 1 kb ladder (Gibco BRL).

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