AN EPIDEMIOLOGICAL STUDY OF GENTAMICIN RESISTANT GRAM NEGATIVE BACTERIA WITH PARTICULAR REFERENCE TO *PSEUDOMONAS AERUGINOSA* AT KING EDWARD VIII HOSPITAL - DURBAN

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SUMMARY

The sources of gentamicin resistant pseudomonads and enterobacteria were studied in detail. A total of 1703 gentamicin resistant gram negative bacilli (GRGNB) isolated from patients, staff and their immediate environment were studied, over a 6 month period. Of these 954 were isolated from clinical specimens obtained from patients and 540 from their immediate environment. A furthur 209 stains were isolated from the staff members who were responsible for the care of these patients.

Pseudomonas aeruginosa; pyocin type 1, phage type F7 and serotype 11 was the commonest isolate. It constituted 24,9% of all isolates in this study. This organism was distributed in all the wards investigated and was isolated throughout the 6 month study period. This strain, therefore, appears to be part of the"resident" flora of King Edward V111 Hospital, for it was found on patients, staff and their immediate environment.

Among the *Enterobacteriaceae, Klebsiella pneumoniae* was the commonest isolate and made up 13,6% of all isolates.

All the isolates obtained in this study were resistant to five or more antibiotics tested (gentamicin, tobramycin, kanamycin, streptomycin, carbenicillin, polymyxin B, amikacin and sisomicin).

Of 310 staff members screened, 25,2% harboured GRGNB on their hands. Among patients the commonest source of GRGNB was stool which yielded 141 (14,8%) of the clinical isolates. Of the environmental sources studied, sinks harboured 87 (14%) GRGNB. The isolates from the environment and staff members were identical to patient strains. The significance of these findings is discussed.

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LITERATURE REVIEW

1.1 Introduction

"It appears that unless drastic measures are taken very soon, physicians may find themselves back in the pre-antibiotic Middle Ages in the treatment of infectious diseases". (1)

One of the greatest achievements in medicine during the past 40 years has been the development and clinical use of antibiotics and chemotherapeutic agents (2). Antibiotics are being widely used at present (sometimes with doubtful indications) not only for man, but also for animals, fish, fruit, vegetables, rice plants and even for honey bees (3,4).

Nature has provided all living organisms with mechanisms to prevent extinction of their species, and in bacteria, this is amply illustrated by the appearance of antibiotic resistant strains.

The problem of bacterial resistance to antibiotics is of serious concern to the clinician. Whether resulting from selective pressure (2,5) due to widespread use of an antibiotic, from mutation or plasmid transfer, bacterial resistance to antibiotics has eroded the values of many of the major antibiotics in some of the common infections; for example penicillin G against staphylococcus (6), streptomycin against enterobacteriaceae (7).

Resistant strains of bacteria have emerged to many of the available antibiotics, yet despite warnings by Professor Mary Barber and others in the 1950's, very few hospitals have bothered to formulate an antibiotic policy and take the necessary measures for the prevention and control of cross-infection by these resistant strains of bacteria.

In the discussion that follows, a brief review on the

mechanisms of bacterial resistance is followed by an attempt to put into perspective the significance of multiple antibiotic resistant gram negative bacteria in clinical practice.

1.2 Aminoglycoside Antibiotics

The aminoglycoside antibiotics in common clinical use include streptomycin, neomycin, kanamycin, gentamicin, tobramycin, netilmicin, sisomicin and amikacin. These drugs are given parenterally, are bactericidal and share similar side effects; but differ in their spectrum of activity. Kanamycin is indicated in serious gram-negative infections in which *Pseudomonas aeruginosa* is not a likely causative agent. Gentamicin, tobramycin and amikacin are effective against a broad spectrum of gram-negative organisms including *P.aeruginosa*.

1.2.1 Mode of Action and Main Pharmacologic Properties

The aminoglycoside antibiotics penetrate the cell wall and cytoplasmic membrane of susceptible microorganisms and act on bacterial ribosomes. They are bound to the 30 s subunit of the ribosome and cause misreading of the genetic code; thus incorrect amino acids are inserted into the peptide chains. Faulty proteins are produced and this results in death of the microorganism (8).

Aminoglycoside antibiotics are poorly absorbed from the gastrointestinal tract, do not penetrate well into the cerebrospinal fluid and are rapidly excreted by the normal kidney.

1.2.2 Mechanisms of Resistance

There are many different mechanisms for antimicrobial drug resistance, and knowledge of the genetic basis for these has increased greatly in recent years.

Natural resistance

The term, intrinsic resistance, is used to indicate natural resistance to antimicrobial agents possessed by the majority of the population of a bacterial species. Such resistance is obvious at the time of the initial introduction of an antimicrobial agent. Several types of bacteria are naturally resistant to polymyxins including many gram-positive bacteria, *Proteus species*, *Serratia species*, *Providencia*, *Pseudomonas cepacia*, *Neisseria meningitidis*, *Neisseria gonorrhoeae* and *Staphylococcus aureus*. *Pseudomonas aeruginosa* resistance to streptomycin is a further example of natural resistance(9,31).

Natural resistance maybe due to lack of penetration of the drug through the cell wall, lack of a suitable cell wall target site or other target receptors in the cell, and susceptibility to naturally produced drug-destroying enzymes that may have existed before the introduction of the drug commercially (31).

Chromosomal resistance

This type of resistance occurs in all bacteria and has been known since the earliest studies on drugs (10). This type of resistance is mediated by genes on the chromosome which have been changed by mutation. Resistance genes are heritable and can be transferred from resistant to sensitive bacteria by transduction, transformation or usually by conjugation (11-13).

Use of a particular antibiotic produces a selective pressure which favours the survival of resistant mutants and their descendants while their sensitive counterparts are inhibited or killed (14). The conditions created by large-scale drug utilization resulted in a rapid increase in the incidence of bacteria with chromosomal resistance (15).

R - Plasmid mediated resistance

Scientists concerned with infectious diseases have now focused attention on structures within bacterial cells. Of these, none are more important practically or theoretically than plasmids (16). A plasmid has been defined as "a replicon that is stably inherited (i.e. readily maintained without specific selection) in an extrachromosomal state. Naturally occuring plasmids of prokaryotes are generally dispensable" (17). These genetic structures determine the inheritance of various properties and lead an independent existance within the bacterial cell. Since they are not essential to cell survival, the cell may gain or lose them without any adverse effect (18).

An R - plasmid is defined by Novick *et al* (17) as one "that carries genetic information for resistance to antibiotics and/or other antibacterial drugs". A bacterium which carries an R - plasmid is able to conjugate with other bacterial cells. When this happens, a duplicate of the R - plasmid is transmitted to the second cell, which thereby acquires all the drug resistance carried on the plasmid and also the potential of transmitting these resistance to still more bacterial cells. The efficiency and ability of different bacterial strains to donate or receive R - plasmid varies (19).

The original discoveries in the field of infectious multiple drug resistance were made in Japan in 1959 (20,21). Soon after this report, transmissible multiple drug resistance was discovered in many other countries throughout the world, including South Africa (22).

The R=plasmids isolated initially in Japan conferred resistance to sulfonamides, chloramphenicol, tetracycline and streptomycin. Subsequently plasmid mediated resistance has been implicated to most of the newer antibiotics introduced including ampicillin, kanamycin-neomycin (23), trimethoprim (24) and gentamicin (25).

By 1965, it was found that many of the common intestinal bacteria isolated from hospitals and other clinical sources carried R - plasmids(16). Although resistance may occur to a single drug, multiple drug resistance seems to be common. Resistance to as many as nine antibiotics, transferred by a single R - plasmid, have been documented (26,27). By transferring plasmids carrying these factors to otherwise harmless bacterial species, the latter could be rendered resistant to the same range of antibiotics as the donors. Since an important feature of R - plasmid transfer is that it is not necessarily species - specific; this implies that a normally harmless intestinal organism such as *Escherichia coli* may transfer multiple resistance to a pathogen such as *Salmonella typhi* (28).

It was generally found that R -plasmids mediate resistance to

higher antibiotic concentrations than that mediated by chromosomal resistance (12,16). R - plasmid resistance is widespread among bacteria and has been reported not only in the Enterobacteriaceae, that is *Salmonella, Shigella, Klebsiella, E.coli, Proteus* and some other gram-negative organisms, such as *P.aeruginosa, Serratia, Yersinia species, Aeromonas* and *Vibrio cholerae* (3,16,29,30) but also in gram positive bacteria (11).

In addition to determining resistance to antibiotics, plasmids are known to carry several other genes. Some of these are known to specify resistance to heavy metals. Nakahara *et al* (32) have reported that the frequency of heavy metal resistance in clinical isolates of *E.coli, Klebsiella pneumoniae, P.aeruginosa* and *Staphylococcus aureus* was the same as or higher than that of antibiotic resistance.

The main practical interest of plasmids in clinical medicine is their ability to transfer antibiotic resistance. The study of plasmids has therefore become of great importance. The widespread distribution of R - plasmids is indicated by various reports on the occurrence of R - plasmids throughout the world. While many of these reports relate to the incidence of R - plasmids in clinical isolates, Datta (33), Moorhouse (34) and Linton *et al* (35) have implicated the normal healthy population as the major reservoir of R - palsmids.

Since their initial discovery in Japan, R - plasmids have been found in many countries throughout the world - for example England (33), U.S.A. (23), Holland (37), Czechoslovakia(38),

South Africa (22) and Switzerland (39) to name a few countries.

The distribution of R - plasmids is not restricted to areas where antibiotics are extensively used. Gardner *et al* (40) examined stool and soil specimens in the Solomon Islands, an area largely untouched by modern civilisation. Of the 40 specimens studied two yielded R - plasmids. Falkow (23) conducted a similar study on a remote population in Australia and isolated only one composite R - plasmid in over 300 strains of *E.coli* isolated from man and his domestic animals, but found that 38% of the strains harboured a sex-factor capable of mobilising non-transferable determinants. Maré (41) however, could find no R - plasmids in 57 strains of enteric bacteria isolated from Kalahari bushmen, although he did isolate drug resistant strains. The negative results in Mare's search for R - plasmids and the detection of R - plasmids in only 2 of 40 specimens in Gardner's study suggest that R - plasmids have a low prevalence in drug-free communities.

Several reports indicate that R - plasmids were present in the pre - antibiotic era. The discovery by Smith (190) of an R - plasmid recovered from a strain of *E.coli* lyophilised in 1946 is often cited. Anderson (16) reported the presence of a transfer factor capable of mobilising resistance genes, in a strain of *Salmonella typhimurium* isolated in 1923. While it is generally accepted that R - plasmids or transfer factors were in existence before the use of antibiotics in medicine and agriculture, it is also accepted that the increasing use of antibiotics is mainly responsible for the rapid emergence of predominantly antibiotic resistant bacterial strains, particularly

among the *Enterobacteriaceae*.

Enzymatic resistance

The most important mode of aminoglycoside resistance by gram-negative bacilli is enzymatic inactivation of the antibiotic. In simple terms, organisms produce substances which modify or destroy the antibiotic. The genetic information for the production of inactivating enzymes is usually borne on R-plasmids (12,43).

Enzymes which modify aminoglycosides fall into three general types: adenylylation (AAD), acetylation (AAC), and phosphorylation (APH). They are further broken down into sub-groups designated by roman numerals (1,11,111, etc) and specified according to which site on the molecule they attack. Table 1.1 summarises the enzymatic mechanisms of inactivation of aminoglycoside antibiotics (44).

It is not entirely clear how these enzymes produce resistance to aminoglycosides. It is argued by some workers that modified aminoglycoside blocks further entry of aminoglycosides into the cell. Others consider inactivation as the resistance mechanism (44).

Table 1.1 Aminoglycoside-modifying enzymes

Enzyme	Modification of							Organisms where found
	Streptomycin	Neomycin B and C	Kanamycin A	Amikacin	Tobramycin	Gentamicin C sissomicin	Netilmicin	
APH(3″)	+	0	0	0	0	0	0	Gram-negative and gram-positive organisms
APH(6)	+	0	0	0	0	0	0	Pseudomonas
APH(3′)	0	+	+	variable	0	0	0	Gram-negative and gram-positive organisms
APH(2″)	0	0	+	±	+	+	±	Gram-positive organisms
AAD(3″)(9)	+	0	0	0	0	0	0	Gram-negative organisms
AAD(6)	+	0	0	0	0	0	0	Staphylococci
AAD(9)	-	0	0	0	0	0	0	Staphylococci
AAD(4′)(4″)	0	+	+	+	+	0	0	Staphylococci
AAD(2″)	0	0	+	variable	+	+	±	Gram-negative organisms
AAC(3)I	0	-	-	-	±	+	±	Gram-negative organisms
AAC(3)II	0	-	±	-	±	+	±	Gram-negative organisms
AAC(3)III	0	+	±	-	+	+	±	Gram-negative organisms
AAC(3)IV	0	+	+	-	+	+	±	Gram-negative organisms
AAC(2′)	0	+	0	0	+	+	+	Providencia
AAC(6′)I	0	+	+	+	+	variable*	+	Gram-negative organisms
AAC(6′)II	0	+	+	+	+	variable*	+	Moraxella
AAC(6′)III	0	+	+	±	+	variable*	+	Pseudomonas
AAC(6′)IV	0	±	+	±	+	variable*	+	Gram-positive organisms

Symbols: + modified: - not modified: ± poorly modified: 0 substituent necessary for modification absent
*Gentamicin C_1 0, gentamicins C_{1A} and C_2 and sissomicin +

1.2.3 Antibiotic Resistance in Clinical Practice

During the last 20 years, there has been an increasing clinical awareness of hospital infections caused by bacteria resistant to several antibiotics available for normal use, and even to some antibiotics in various stages of development.

Antibiotic resistant bacterial populations arise in man and animals by the use of antibiotics whether for therapy, growth promotion or prophylaxis (45). It has been contended by Kelly *et al* (46), Smith (42), Guinee (37) and Lebek (39) that widespread usage of antibiotics in animal feeds has played a significant role in encouraging the emergence of resistant bacteria while other workers like Walton (47) and Aronson (36) seem to suggest that the indiscriminate use of antibiotics in human medicine provides a major selective force for the emergence of resistant strains.

To appreciate the potential impact of antibiotics on bacteria, one should be aware of the quantities of antimicrobial drugs produced annually. In the United States of America the annual production of antibiotics rose from 2,2 million kilograms in 1960 to 8,2 million kilograms in 1970, an increase of almost 400% (48). In Britain, estimates for 1967 (49) indicate that 360 thousand kilograms of antibiotics were used. The human bacterial flora is subjected to this tremendous antibiotic selection pressure primarily under conditions of therapy which makes the hospital environment very important.

Isenberg and Berkman (5) have claimed that in an average 300 bed hospital, the process of clearing the needle by expelling a small portion of the syringe contents prior to injection,

adds 15 to 30 litres of high potency antimicrobial agents to the environment annually.

It is generally agreed that antibiotics are vital in the treatment of diseases but the indiscriminate use of antibiotics by members of the medical profession has been questionable. Aronson (36) sites a survey in which the justification for clinical use of antibacterial drugs was evaluated. Of 340 patients receiving antibacterial drugs, 13% of the therapies were judged rational, 22% questionable and 66% irrational. He also questions the concept of giving antibacterial drugs to be on the "safe side" and states that "the routine prophylactic use of antibacterial drugs in simple surgical procedures is now generally accepted as misuse of this group of drugs."

It must be expected therefore, that organisms isolated from the hospital environment would be drug resistant. The nature of this selection pressure changes with the introduction of new drugs and the decrease in use of some older drugs (5).

As each new antibiotic has been introduced in the hospitals, resistant bacterial strains have emerged (50-59). A strain of such an organism may colonize the hospital environment and cause nosocomial (hospital acquired) infection in a succession of patients for months or even for years. Over the years some antibiotics have ceased to be useful for treating infections caused by certain organisms because these bacteria readily developed resistance to the antibiotics. This has been the case with penicillin G in the treatment of

Staphylococcus aureus. *S. aureus* has undergone a number of changes in its susceptibility pattern. Widespread use of penicillin G has led to increasing proportions of penicillinase producing *S. aureus* such that recommended therapy for suspected staphylococcal infections now consists of a penicillinase resistant agent (e.g. cloxacillin, methicillin), even if the infection is community acquired (6). In 1979, 84,5% of *S. aureus* strains were resistant to penicillin at the Foothills Hospital in Calgary, Canada (7).

Resistance to aminoglycoside antibiotics has commonly followed clinical use of these agents (60). Since 1964 gentamicin has been used extensively for the treatment of severe infections caused by *Enterobacteria, Pseudomonas species* and related gram negative bacilli. Reported hospital outbreaks of infection by gentamicin-resistant organisms have been due to *Pseudomonas* (52-55, 63, 69, 73, 85-89,91), *Proteus* (56, 57, 80, 82, 83, 85, 87-89), *Providencia* (58, 80, 82, 83), *Klebsiella* (50, 59, 70, 74-85, 87-89), *Serratia* (61, 62, 66-68, 72, 80, 82, 85, 91), *Acinetobacter anitratus* (64, 65, 71, 87), *Citrobacter* (74, 82, 83, 88, 89), *Escherichia coli* (74, 82, 83, 85, 87-89), and *Enterobacter species* (80, 82, 83, 87-89).

P. aeruginosa is an opportunist pathogen of increasing concern in hospitals (54, 55, 69, 91, 284-286). The frequency of gentamicin resistance among strains of *P. aeruginosa* has been reported by several groups (52-55, 63, 69, 73, 85-89, 91, 287-289). This organism is responsible for serious infections of burns and wounds. It is one of several agents causing urinary infections in patients who have some structural or neurogenic abnormality in the urinary tract, and is

sometimes responsible for pneumonia, meningitis, eye infections and septicaemia, especially in debilitated hosts (7).

The respiratory tract of patients with tracheostomy often becomes colonized with *P. aeruginosa*. In many cases this appears to have no pathological effect, in others however, illness and death due to infection of the broncho-tracheal tree takes place and such infection is recognised to be significant hazard of tracheostomy (134).

Urinary tract colonization/infection is one of the commonest type of hospital-acquired infection and is particularly associated with catheter drainage. Micro-organisms may enter through the lumen of the catheter or between the catheter and the wall of the urethra. *P. aeruginosa* is a common aetiological agent and owing to its pronounced resistance to antibiotics because of decreased cell wall permeability to antimicrobials, the choice of an appropriate drug is limited.

In one hospital in Los Angeles 20% of clinical isolates of *P. aeruginosa* and 50% of *Serratia marcescens* isolates were resistant to gentamicin (73) and 20% of gram-negative bacilli isolated from sputum and blood in a group of hospitals in Japan were resistant to gentamicin (135).

An outbreak of nosocomial urinary tract infections caused by aminoglycoside-resistant *Pseudomonas aeruginosa* was described by Smith *et al* (69). All 14 isolates of *P. aeruginosa* were generally resistant to many other antibiotics as well as gentamicin, tobramycin, amikacin, streptomycin and kanamycin. Resistant infection was associated with longer hospital stay,

and more frequent corticosteroid usage and prior antibiotic administration. The mortality rate for this outbreak was 25 per cent.

Reports in both the clinical and microbiological literature have described *P. aeruginosa* strains resistant to both gentamicin and carbenicillin (86, 87, 136, 137).

The emergence of gentamicin and carbenicillin-resistant *P. aeruginosa* in a hospital environment was described by Gaman *et al* (86). During a 6 month period, twice weekly cultures were obtained from all patients treated with either gentamicin or carbenicillin and from all patients with a positive culture of *P. aeruginosa*. *P. aeruginosa* was isolated from 238 patients. In 11 other patients, serial cultures revealed the emergence of resistance to gentamicin. All but one of these resistant isolates occurred in patients treated with gentamicin. Gentamicin-resistant *P. aeruginosa* emerged significantly more often in patients treated with gentamicin than in those who did not receive gentamicin. Carbenicillin-resistant *P. aeruginosa* emerged in four of 14 patients treated with carbinicillin. Seventeen of the 238 patients were infected de novo with carbenicillin-resistant *P. aeruginosa*. Carbenicillin-resistant *P. aeruginosa* emerged significantly more often in patients treated with carbenicillin than in those who did not receive carbenicillin. No eivdence was found of in-hospital spread of resistant *P. aeruginosa*.

Richmond (99) has cited in detail the literature relating to the emergence of carbenicillin resistant *P. aeruginosa* in burns patients. Detailed molecular studies on plasmids from

Pseudomonas and *Klebsiella* strains suggested that the transfer of the R-plasmid, first observed in *Klebsiella* and subsequently in *P. aeruginosa*, had occurred from the former to the latter via faecal contamination of burn wounds.

Pseudomonas cepacia was first reported in the literature by Burkholder (100) who described it as a plant pathogen associated with a bacterial rot of onion bulbs. The basic description of morphology and growth characteristics was later considerably expanded, and it was recognized that *P. cepacia* was the most nutritionally versatile of the pseudomonads, being able to utilize a wide range of organic compounds as an energy source (101). Natural sources of *P. cepacia* appear to be the soil and water, but increasingly common is the clinical isolation from the hospital environment.

In spite of its ubiquitous distribution in soil and water (102) this organism rarely causes infection outside the hospital environment. Two general exceptions to this have been reported: *P. cepacia* endocarditis occasionally affects intravenous drug abusers (103) and *P. cepacia* dermatitis has been observed in the toe webs of troops, commonly known as 'trench foot' after prolonged immersion in contaminated water (102).

Within the hospital environment wet surfaces, water and aqueous solutions appear to be the most common sources of the organism. Fortunately its level of invasiveness and virulence is low and the only population susceptible to infection appears to be those hospital patients with serious underlying diseases. Patients requiring instrumentation, particularly with urinary problems are a particularly high risk group (104).

P. cepacia is a potentially dangerous, nosocomial pathogen and has been implicated in a wide range of hospital epidemics and sporadic infections. These have included infections of the respiratory tract (105); of operation wounds (106); of the urinary tract (104); and of the heart (103, 107). In addition it has been implicated in pneumonia (104), septic arthritis (108), peritonitis (109) and many generalized infections have been reported (104-106, 110).

Infections have arisen from the use of contaminated anaesthetics (111), detergents (112), disinfectants (113), antiseptics(106, 114), saline solutions (110) and the water baths used for warming blood before blood infusions (115). It is therefore apparent that the organism is fairly widespread throughout the hospital environment. However, a common link in most cases seems to be a contaminated water supply and this observation is frequently related to the ability of *P. cepacia* to multiply in distilled water (116), and to resist inactivation by most commonly used disinfectants (117). In fact, it can multiply as quickly in distilled water as in tryptone soy broth (116).

This organism has a high intrinsic resistance to most antibiotics (118). Beyond doubt, infection with *P. cepacia* is encouraged by the use of antibiotics, to which they are mainly resistant (119). In a study of the antibiotic susceptibility of various clinically isolated strains of pseudomonads, over 50 per cent of the *P. cepacia* strains were resistant to all 12 of the tested antibiotics and the majority were resistant to 11 out of the 12 (120). Even though infection of a patient with *P. cepacia* can be a problem, colonization is far more

common.

The risks of cross-infection with gentamicin-resistant enterobacteria appear to be greater for *Klebsiella* species than for *E. coli*, *Citrobacter* species or *Enterobacter* species. This may be due to a particular ability of *Klebsiella* to become established in the urinary and intestinal tracts (83, 121) and to its ability to survive well on skin and on dry surfaces (122).

Hart (83) describes an outbreak of nosocomial gentamicin and multiple-resistant enterobacteria at the Royal Liverpool Hospital, Liverpool. During a two year period from 1979-1981, gentamicin and multiple-resistant strains of enterobacteria were isolated from 260 patients. All isolates showed a similar resistance pattern and consisted of *E. coli*, *Citrobacter*, *Enterobacter*, *Proteus*, *Providencia* and *Klebsiella* species. *Klebsiella* was the commonest isolate and the organism responsible for cross-infection. The 32 different capsular serotypes encountered in this outbreak represent a far greater diversity of gentamicin-resistant *Klebsiella* than has been reported previously. The majority of infections followed manipulation of the urinary tract and were often associated with in-dwelling urinary catheters or were in immunosuppressed patients. The inanimate environment in close contact with patients was infrequently contaminated and then only with *Klebsiella* species. The bed pan macerators occasionally released an aerosol containing gentamicin-resistant *Klebsiella*. The majority (83%) of sluice sinks were heavily contaminated with many different

gentamicin-resistant enterobacteria including 22 different *Klebsiella* capsular serotypes. All the gentamicin-resistant enterobacteria were also resistant to tobramycin, netilmicin, kanamycin, streptomycin, spectinomycin, sulphamethoxazole, trimethoprim, tetracycline, ampicillin, cepahloridine and chloramphenicol.

Selden *et al* (121) undertook a prospective study of patients admitted to a hospital in which there was endemic nosocomial infection with multidrug-resistant *Klebsiella*. The role of intestinal colonization was evaluated. Of 31 patients who became intestinal carriers of multidrug-resistant *Klebsiella* during hospitalization, 14 subsequently became infected by the same serotype. Of 101 patients who did not become intestinal carriers of *Klebsiella*, only 11 were infected by that organism. Antibiotic therapy was shown to be predisposing to intestinal colonization with *Klebsiella* and to exert a selective pressure in favour of multidrug-resistant *Klebsiella*. All the isolates of *Klebsiella* were generally resistant to many other antibiotics as well as gentamicin, streptomycin and kanamycin. Gastrointestinal acquisition and carriage of *Klebsiella* by patients may be an important intermediate step in the development of nosocomial *Klebsiella* infection and may also serve to perpetuate a significant reservoir of organisms within the hospital.

Gentamicin resistance has emerged since 1971 among gram negative bacilli isolated at the University of Virginia Hospital (123). Of 9,169 gram-negative bacilli isolated in 1971, 0,8% were resistant to gentamicin. In 1975, 7,7% of 7,817 isolates were gentamicin resistant. Since 1974, gentamicin-resistant gram-negative bacilli have accounted for 20% of nosocomial bacteremias. In 1974-1975,

34 episodes of gentamicin-resistant bacteremia occurred. The organisms isolated were *Klebsiella* species, *P. aeruginosa, Enterobacter* species, *Serratia marcescens, Flavobacterium* species, *Proteus* species, *Acinetobacter* species, *E. coli* and *Citrobacter freundii*. All but one of the bacteremic patients had received prior antibiotics, and 19 of 34 episodes were preceded by gentamicin therapy.

In another study (77) more than 241 patients became colonized with a strain of gentamicin-resistant *Klebsiella aerogenes,* capsular serotype K2, resistant to most antibiotics. Urinary tract infection was the most common clinical manifestation but bacteremia and, occasionallly, infections of other sites were encountered. The main reservoirs of the epidemic *Klebsiella* were the gut, urine and skin of colonized patients. Likely vehicles for spread were the hands of staff, and contaminated bedpans and urinals.

Casewell and Talsania (90) describes a microbiological study of multiple antibiotic resistance and capsular types of gentamicin-resistant *Klebsiella aerogenes.* One hundred and eight epidemiologically distinct strains of gentamicin-resistant *Klebsiella aerogenes* were selected from 404 patient-isolates sent from 12 hospitals in six countries. Serotypes K2, K17 and K21 were commonest and together accounted for 33,3% of strains. Resistance to many antibiotics in addition to gentamicin was common; 99 strains were resistant to at least 10, and 31 to at least 15, of the 24 antimicrobial agents tested. Of the 108 strains, 100

were resistant to streptomycin, 96 to kanamycin, 85 to tobramycin, 81 to neomycin and 4 to amikacin. All strains were resistant to carbenicillin and ampicillin.

Serratia marcescens is another organism that is well established as an important cause of nosocomial infection, spread by various routes (124). Bullock *et al* (66) describes five geographically separate outbreaks of hospital acquired infection caused by gentamicin-resistant strains of *Serratia marcescens* which occurred in southwest England. The patients affected were in wards for general or urological surgery, or in neurosurgical, cardiothoracic or general intensive therapy units. Asymptomatic colonization was more common than symptomatic infection, although deaths and serious infections occurred. Besides gentamicin, most strains were resistant to many currently available antibiotics like tobramycin, sisomicin, netilmicin, neomycin, kanamycin and streptomycin. Control of spread of the bacteria proved to be difficult.

Platt and Sommerville (61) describes a twelve months survey which yielded 119 isolates of *Serratia* species from 58 patients. Ninety-one per cent of the patients were compromised, 86% had undergone recent surgery and 79% had received antimicrobial therapy within 14 days prior to serratia isolation. The principal sources of the isolates were the intensive care and peripheral vascular units. Fifty-seven per cent of the isolates were resistant to carbenicillin and 30% resistant to gentamicin. Gentamicin resistance was exclusively plasmid-mediated whereas resistance to carbenicillin was equally divided between plasmid-mediated and chromosomal mechanisms.

The main natural habitat of *Acinetobacter anitratus* is soil or water (93). It has also been isolated from human skin (94) including the hands of hospital staff (95). Laboratory isolates from clinical specimens usually reflect colonization rather than infection (98) but serious, sometimes fatal, opportunistic infections have been reported, including septicaemia, endocarditis, meningitis, and pneumonia (96, 97). Increased colonization and infection caused by *A. anitratus* in hospitals (97) has sometimes been reported as epidemic spread (95).

French *et al* (64) reports a hospital outbreak of antibiotic resistant *Acinetobacter anitratus* which was recognized by its characteristic biotype and non-transferable resistance to 18 antibiotics. The organism was sensitive to nalidixic acid, colistin, tobramycin (M I C 1mg/l) and amikacin (M I C 4mg/l), but was otherwise multiple-resistant with M.I.C's of more than 128 mg/l of ampicillin, carbenicillin, azlocillin, mezlocillin, cephaloridine, cephalothin, cephalexin, cefoxitin, cefsulodin, streptomycin, kanamycin and gentamicin. The organism was also resistant to cefotaxime (M I C 16 mg/l), cefuroxime (M I C 64 mg/l), trimethoprim, sulphamethoxazole, tetracycline, and nitrofurantoin. The epidemic strain was isolated from 40 patients in six wards. Most isolates were from the urine of catheterized male patients in a urological ward, some of whom had skin but not rectal colonization. Elimination of environmental contamination did not alter the course of the outbreak, and respirators were not implicated. Transmission of the epidemic strain from patients' skin to staff hands was demonstrated experimentally, and of 38 staff working in affected

wards, 11 had positive hand cultures. Two microbiologists investigating the outbreak also became hand carriers. Prompt identification of new cases and closer attention to staff hand washing led to disappearance of the epidemic strain.

Holton (65) makes a report of a further hospital outbreak caused by a multi-resistant *Acinetobacter anitratus*. Over a period of two years there were 58 separate isolates of *Acinetobacter* species at the Middlesex Hospital, London. Most isolates (20) were from sputum, 18 from wound swabs, 14 from urine, five from bile and one from blood culture. Six of the isolates were *Acinetobacter lwoffii*, the remainder were *A. anitratus*. The isolates were generally resistant to many other antibiotics as well as carbenicillin and gentamicin. All isolates were sensitive to nalidixic acid, tobramycin and amikacin. The source of the organism was not established.

Resistance to administration of streptomycin and kanamycin has become so prevalent among gram-negative bacilli in many hospitals that the clinical usefulness of these drugs has been severely impaired (67, 88-90, 92). Organisms resistant to gentamicin, tobramycin and amikacin have also been isolated (69, 88-90). Organisms with these high resistance patterns have been isolated with increasing frequency at many hospitals. The emergence and existence of these organisms is of great epidemiologic importance.

Hospital Cross-infection by Antibiotic Resistant Bacteria Some Clinical Reports on Emergence and Suppression.

Hospitals all over the world have reported sporadic incidents

of cross-infection with organisms resistant to aminoblycosides and other antibiotics. Some clinical reports have mentioned the possible source and control of these resistant organisms.

Falkiner *et al* (126) reported an outbreak of gentamicin and tobramycin-resistant *P. aeruginosa* infections in a surgical ward over a 3 month period. They found that resistant *P. aeruginosa* strains with similar serological, phage, and pyocine types cultured from the urine of six patients were also found on urine bottles, bed pans and hands of attendant staff. From their survey they concluded that inadequate disinfection played a major role in cross-infection.

Bulger *et al* (127) surveyed gram negative bacteria in a hospital ward during a 7 year period and observed a decline in the incidence of resistance to several antibiotics among strains of *E. coli* and *Enterobacter - Klebsiella*. They attributed these findings in part to conservative and selective use of antibiotics, and in part to infection control.

Ridley *et al* (128) found that the rise and fall in incidence of antibiotic-resistant *Staphylococcus aureus* during an 11-year period correlated with consumption of antibiotics.

Price and Sleigh (129) described an epidemic of infection with *Klebsiella aerogenes* in a neurosurgical unit : although the epidemic was not curtailed by radical measures for prevention of cross-infection, complete discontinuation of antibiotics led to disappearance of the epidemic strain.

Noriega *et al*(130) found that resistance to gentamicin increased

abruptly among nosocomial isolates of *Klebsiella* and *Enterobacter* at the New York Veterans Administration Hospital in 1973 and 1974. They also observed that gentamicin-resistance declined rapidly after use of this antibiotic was restricted and increased several months after restrictions were removed. They concluded from this study that "..... brief intermittent restrictions on use of gentamicin may be sufficient to maintain a survival advantage of sensitive strains. Since either amikacin or combined polymyxin-sulphonamide-trimethoprim is available for treatment of gentamicin-resistant infection, a programme in which these agents are rotated may control resistance to each while providing reliable activity against most gram-negative species. This approach appears to be more rational than the unlimited use of all agents during the same period."

The emergence of gentamicin-resistant *P. aeruginosa* during topical therapy has been reported. In 1967, Muller in Germany reported that 4 of 50 patients treated with topical gentamicin developed gentamicin-resistant organisms during treatment. When usage of gentamicin was stopped for one month, resistant strains were not detected. (131). Snelling *et al* (132) have also reported colonization with gentamicin-resistant *Pseudomonas* in 4 of 24 patients with burns treated with topical gentamicin.

Shulman *et al* (54) reported an outbreak of colonization with gentamicin-resistant *P. aeruginosa*, pyocine type 5 in the burn service at Grady Hospital. In their study the resistant *Pseudomonas* occurred frequently with topical therapy although topical gentamicin was not applied daily or in an uninterrupted

fashion. The data presented suggests that gentamicin-resistant *Pseudomonas* may appear in a setting of widespread usage of topical gentamicin.

A gentamicin resistant strain of *Klebsiella aerogenes* was isolated from the urine of 17 patients out of 237 admitted to a male urological ward (125). The factors most frequently associated with *K. aerogenes* in the urine were catheterisation and antibiotic therapy. Often the epidemic strain (type K 16) was found not only in the patients' faeces but also on more remote skin sites such as hands, knees, groin and the umbilicus. Barrier nursing of colonized patients, stringent staff hand-washing with chlorhexidine, and the use of disposable aprons contained the outbreak.

Casewell and Phillips (74) found *Klebsiella* species contaminating the hands of 17% of the staff of an intensive care unit. These strains were related to serotypes infecting or colonizing patients in the ward on the same day. Handwashing with chlorhexidine hand cleanser reliably gave 98-100% reduction in hand counts. The introduction of routine handwashing by staff before moving from one patient to the next was associated with a significant and sustained reduction in the number of patients colonised or infected with *Klebsiella* species.

Over a three-month period, *Proteus rettgeri* infections occurred in ten patients in one general medical ward at the Presbyterian St. Luke's Hospital, Chicago (57). This organism was resistant to all antimicrobials tested including tobramycin, gentamicin, kanamycin, carbenicillin, ampicillin, cephalothin, tetracycline,

chloramphenicol, polymyxin and rifampicin. Clinical illness from this organism was directly related to local or systemic host deficiencies. Apart from the patients, no reservoir was shown by culturing personnel, catheter kits, the hospital environment, and the stools and urine of every patient on the ward. Probable spread was by contact through the intermediary of hospital personnel. The outbreak was controlled by having staff use disposable gloves and by contact isolation of patients.

From these various studies it is apparent that indiscriminate and continued use of an antibiotic, and poor hygiene, coupled with inadequate hospital infection control, are the major factors in allowing the emergence and dissemination of antibiotic resistant bacteria.

This study was undertaken at King Edward VIII Hospital (K E H) to :-

(1) Define the extent and nature of bacterial resistance to antibiotics.

(2) Identify the sources of such organisms by investigating :-

  (a) Staff at the hospital
  (b) Patients harbouring such resistant organisms
  (c) The environment of these patients

(3) Determine the role of these organisms in hospital cross-infection.

King Edward VIII Hospital is attached to the Medical Faculty of the University of Natal. Still the main teaching hospital,

this must be one of the busiest in the world. It is a 2000 bed general hospital with approximately one million patients being seen at the out patient and casualty departments annually, and a 100 000 admissions recorded yearly. Despite this accommodation, it is always full and often overcrowded. Its main task is to serve the Africans and Indian communities of a metropolitan area which has more than a million people.

MATERIALS AND METHODS

2.1 Patient Selection and Collection of Specimens

Gentamicin resistant strains of gram negative bacilli were obtained from clinical specimens processed routinely at the Bacteriology Laboratory of King Edward VIII Hospital, Durban. Patients found to be infected by gentamicin-resistant enterobacteria and pseudomonads were screened for carriage of these organisms as soon as possible after the infection was detected. This was done over a six month period.

Each patient was screened by means of rectal swabs, oral swabs and hand swabs; in addition some of the patients were also screened for vaginal carriage, nasal carriage and carriage at skin sites such as the groin, umbilicus and axilla.

In the case of patients with burns, two representative areas of the burned surface were sampled. Other sites sampled included wounds, abscesses, ears and the trachea (in the cases of tracheostomy). Specimens of urine, stool, sputum and blood cultures were also taken where appropriate. Fomites in the vicinity of the patient were also sampled. In the case of patients dying, post mortem specimens including heart blood and lung aspirates were taken within half-hour of death.

2.2 Screening of Staff Members

Swabs were taken from the hands, throat, anterior nares and rectum of all members of the staff on duty, including nurses, doctors and cleaners, treating such patients directly or

indirectly.

Staff uniforms, gowns, aprons and overshoes were also sampled with contact plates.

2.3 Environmental Surveillance

In order to define the magnitude of environmental contamination by gentamicin resistant gram negative bacilli; swabs were collected from the floors, walls and ventilator equipment. Contact plates were taken from the bed sheets, mattresses, gowns and plastic aprons. Food, medicines and soaps were also sampled. Samples from sink surfaces, sink traps, water taps, nasopharyngeal and urinary catheters were also taken. In addition urinals, bed-pans, nail brushes, baths, toilet seats, ward sluice rooms, kitchens and other miscellaneous surfaces were sampled. Stock and 'in use' disinfectant solutions were also cultured.

Since respiratory equipment has frequently been described as an important reservoir of *Pseudomonas* contamination (134,138,139) such equipment was carefully monitored. Samples were taken in this series from expiratory and inspiratory tubing, humidifiers, Y-pieces and tracheal connectors, including condensate, water traps, spirometers, humidifier reservoirs, catheter mounts, positive and negative bellows and air effluent.

Settling plates containing blood agar were also placed at various sites in the wards to identify large droplet or particle fallout from the air.

2.4 Sampling Methods

All dry areas were sampled with sterile absorbent cotton wool

swabs moistened with sterile peptone water. On all other surfaces dry sterile swabs were used.

Specimens from burns, wounds and abscesses were taken with dry, sterile cotton wool swabs.

Urine, stool and sputum specimens were collected in sterile universal containers.

Blood cultures were taken using the Vacutainer Brand 50 ml Culture Tube (Becton-Dickinson).

Hands were sampled using finger print impressions on blood agar plates containing 5 µg per ml gentamicin. Uniforms, gowns, plastic aprons, bed linen, mattresses and dressings were sampled with contact plates containing blood agar with 5 µg per ml gentamicin.

Heart blood and lung aspirates were collected into 5 mls nutrient broth.

2.5 Bacteriological Methods

All specimens were plated onto MacConkey and Blood agar containing 5 µg per ml gentamicin. Blood cultures and lung aspirates were incubated aerobically at 37°C for 48 hours before being plated onto the above mentioned plates. The plates were incubated aerobically at 37°C and examined after 24 hours and 48 hours. All gram negative bacilli were initially checked for gentamicin resistance by the controlled disc diffusion method of Stokes (140). These gentamicin resistant gram negative bacilli were immediately sub-cultured onto nutrient agar slopes containing 10 µg per ml gentamicin for further

tests which included :-

2.5.1 Identification of the organism.

2.5.2 Determination of antibiotic susceptibility pattern.

2.5.3 Determination of minimum inhibitory concentrations (MIC).

2.5.4 Pyocin typing of *P. aeruginosa*.

2.5.5 Phage typing of *P. aeruginosa*.

2.5.6 Serological typing of *P. aeruginosa*.

2.5.1 Identification of the Organisms

Strains were identified according to the following tests using the criteria of Wahba and Darrell (141), Phillips (142), King and Phillips (143), Cowan and Steel (144), Edwards and Ewing(145)and MacFaddin (146).

Pseudomonas aeruginosa

i) Classical colonial appearance - typical flat irregular colonies.

ii) Production of diffusible green, yellow or blue pigment.

iii) Strong smell of trimethylamine - grape-like odour.

iv) Triple sugar iron agar (TSI) alkaline-non-fermentation-metallic sheen on slant.

v) Sellers Agar (BBL): Slant - blue or green
Butt - $\pm$ yellow (butt is never green if the slant is blue).

vi) Motility : Positive

vii) Oxidase : Positive

viii) Fluorescence in U.V. light : Positive

ix) Growth at $5^{o}C$: Negitive

x) Growth at $42^{o}C$: Positive

xi) Simmons' citrate : Positive

xii) Carbonhydrates (Hugh & Leifson base), acid from :

glucose : Positive

lactose : Negative

maltose : Negative

salicin : Negative

sucrose : Negative

xiii) Starch hydrolysis : Negative

xiv) Nitrate reduced to nitrite : Positive

xv) Arginine dihydrolase : Positive

xvi) Urease : Positive

Pseudomonas cepacia

i) Triple sugar iron agar : alkaline, non-fermentation.

ii) Motility : Positive

iii) Oxidase : Positive

iv) Fluorescence in U.V. light : Negative

v) Growth at $5^{o}C$: Negative

vi) Growth at $42^{o}C$: Positive

vii) Simmons' citrate : Positive

viii) Carbohydrates (Hugh & Leifson base), acid from :

glucose : Positive

lactose : Positive

maltose : Positive

salicin : Positive

sucrose : Positive

ix) Starch hydrolysis : Negative

x) Nitrate reduced to nitrite : Positive

xi) Urease : Positive

xii) Arginine dihydrolase : Negative

Pseudomonas stutzeri

i) Triple sugar iron agar: alkaline, non-fermentation.

ii) Motility : Positive

iii) Oxidase : Positive

iv) Fluorescence in U.V. light : Negative

v) Growth at $5^{o}C$: Positive

vi) Growth at $42^{o}C$: Positive

vii) Carbohydrates (Hugh & Leifson base), acid from :

glucose : Positive

lactose : Negative

maltose : Positive

salicin : Negative

sucrose : Negative

viii) Starch hydrolysis : Positive

ix) Nitrate reduced to nitrite : Positive

x) Urease : Negative

xi) Arginine dihydrolase : Negative

xii) Simmons' citrate : Positive

Alcaligenes faecalis

i) Triple sugar iron agar: alkaline, non-fermentation

ii) Sellers agar : blue slant, green butt

iii) Motility : Positive

iv) Oxidase : Positive

v) Growth at $42^{o}C$: Positive

vi) Simmons' citrate : Positive

vii) Malonate : Positive

viii) Aesculin hydrolysis : Negative

ix) Indole : Negative

x) Urease : Negative

Flavobacterium meningosepticum

i) Triple sugar iron agar : alkaline, non-fermentation

ii) Motility : Negative

iii) Oxidase : Positive

iv) Pigmentation : $22^{o}C$ beige to yellow pigment
$37^{o}C$ light yellow

v) Growth at $42^{o}C$: Negative

vi) Indole : Positive

vii) DNase : Positive

viii) Aesculin hydrolysis : Positive

ix) Urease : Negative

Acinetobacter anitratus var *calcoaceticus*

i) Triple sugar iron agar : alkaline, non-fermentation

ii) Sellers agar : yellow band between blue slant and green butt.

iii) Oxidase : Negative

iv) Growth at $42^{o}C$: Positive

v) O-F glucose (Hugh & Leifson) : Oxidative

Escherichia coli

i) T.S.I. : slant - acid
butt - acid and gas

ii) Motility : Positive

iii) Catalase : Positive

iv) Oxidase : Negative

v) Citrate : Negative

vi) Indole : Positive

vii) Urease : Negative

viii) Hydrogen sulphide (H_2S) from TSI : Negative

Providencia stuartii

i) T.S.I. : slant-alkaline
butt-acid, no gas

ii) Motility : Positive

iii) Catalase : Positive

iv) Oxidase : Negative

v) Citrate : Positive

vi) Malonate : Negative

vii) Adonitol : Negative

viii) Arabinose : Negative

ix) Inositol : Positive

x) Rhamnose : Negative

xi) Indole : Positive

xii) Urease : Negative

xiii) H_2S from TSI : Negative

xiv) Arginine dihydrolase (AD) : Negative

xv) Lysine decarboxylase (LD) : Negative

xvi) Ornithine decarboxylase (OD): Negative

xvii) Phenylalanine (PD) : Positive

Proteus morganii, Proteus mirabilis and Proteus rettgeri

	TEST	*P. morganii*	*P. mirabilis*	*P. rettgeri*
i	T.S.I. slant	Alkaline	Alkaline	acid
	butt	acid & gas	acid & gas	acid & gas
ii	Motility	Positive	Positive	Positive
iii	Catalase	Positive	Positive	Positive
iv	Oxidase	Negative	Negative	Negative
v	Citrate	Negative	Positive	Positive
vi	Malonate	Negative	Negative	Negative
vii	Adonitol	Negative	Negative	Positive
viii	Arabinose	Negative	Negative	Negative
ix	Inositol	Negative	Negative	Positive
x	Rhamnose	Negative	Negative	Positive
xi	Indole	Positive	Negative	Positive
xii	Urease	Positive	Positive	Positive
xiii	H_2S from T.S.I.	Negative	Positive	Negative
xiv	AD	Negative	Negative	Negative
xv	LD	Negative	Negative	Negative
xvi	OD	Positive	Positive	Negative
xvii	PD	Positive	Positive	Positive

Enterobacter cloacae, Enterobacter aerogenes, Klebsiella aerogenes and *Klebsiella pneumoniae*

TEST	*E. cloacae*	*E. aerogenes*	*K. aerogenes*	*K. pneumoniae*
i) TSI slant	acid	acid	acid	acid
butt	acid & gad	acid & gas	acid & gas	acid & gas
ii) Motility	Positive	Positive	Negative	Negative
iii) Catalase	Positive	Positive	Positive	Positive
iv) Oxidase	Negative	Negative	Negative	Negative
v) Citrate	Positive	Positive	Positive	Positive
vi) Malonate	Positive	Positive	Positive	Positive
vii) Adonitol	Positive	Positive	Positive	Positive
viii) Arabinose	Positive	Positive	Positive	Positive
ix) Inositol	Negative	Positive	Positive	Positive
x) Rhamnose	Positive	Positive	Positive	Positive
xi) Methyl Red test	Negative	Negative	Negative	Positive
xii) Voges-Proskauer test	Positive	Positive	Positive	Negative
xiii) Indole	Negative	Negative	Negative	Negative
xiv) Urease	Positive or Negative	Positive or Negative	Positive	Positive
xv) H_2S from TSI	Negative	Negative	Negative	Negative
xvi) AD	Positive	Negative	Negative	Negative
xvii) LD	Negative	Positive	Positive	Positive
xviii) OD	Positive	Positive	Negative	Negative
xix) PD	Negative	Negative	Negative	Negative

2.5.2. Determination of Antibiotic Susceptibility Pattern

Essentially two categories of defined disc susceptibility tests have emerged. These are :-

(i) The Kirby-Bauer (147) and Ericsson methods (148) and

(ii) The Comparative and Stokes methods (140).

(i) The Kirby-Bauer and Ericsson methods

The Kirby-Bauer method (or modifications thereof) is widely used in the United States, and is the technique recommended by the Food and Drug Administration (149) and the National Committee for Clinical Laboratory Standards Sub-Committee on Antimicrobial Susceptibility Testing(150) It is also the method preferred by the South African Institute for Medical Research.

This method specifies that only Mueller-Hinton medium can be used. Inoculum is standardised by adjusting the density to that of a barium-sulphate standard and plates are inoculated by dipping a swab into the suspension and streaking it across the medium; this usually gives a confluent growth. Discs of relatively high antibiotic content are used. After applying sensitivity discs, the plates are incubated immediately or within 15 minutes of preparation. Following overnight incubation at $37^{o}C$, zone diameters are measured and compared by reference to a table. The zone measurements are not reported, only the interpretations resistant, sensitive or intermediate.

The Ericsson method is commonly used in Sweden and is a development of the method recommended by the WHO-sponsored International Collaborative Study of Sensitivity Testing.

This method also specifies the medium to be used but gives alternatives, Mueller-Hinton medium or PDM medium (AB Biodisk, Sweden). Inoculum is standardised to a density that gives semi-confluent growth. The medium is inoculated by flooding the plates. Plates are dried for 30 minutes at 37°C before discs are applied. The antibiotics are allowed to diffuse for 30 minutes at room temperature before plates are incubated overnight. The size of the inhibition zones are then measured and compared with Regression-Slope graphs prepared from testing large numbers of organisms. Reports may then be given in terms of sensitivity/resistance or as minimum inhibitory concentration values.
Both the Kirby-Bauer and Ericsson methods attempt to control variations by rigid standardization of all aspects of the procedure.

(ii) The Comparative and Stokes methods.

These methods are commonly used in British laboratories in a variety of modifications of the methods described by the Association of Clinical Pathologists (140). Unlike the Kirby-Bauer technique, which relates zone diameter to minimum inhibitory concentration, these techniques compare the zone of inhibition in respect of a test organism with that of an antibiotic-susceptible control. The Kirby-Bauer method requires rigid standardization of the technique, whereas these methods allow some variation of technique and medium.

The Comparative Control Technique as described below was used in this study.

Plate preparation :

Approximately 25 ml of Oxoid Iso-Sensitest agar was poured into an 85 mm-diameter petri dish, and allowed to set on an even

horizontal surface. The plates were stored at $4^{o}C$ and used within seven days.

Bacterial inoculum

The inoculum was prepared by emulsifying portions of three to five bacterial colonies in fifteen mls of sterile distilled water. This gave semiconfluent growth after 16-20 hours (overnight) incubation. The control organisms were prepared in a similar manner.

Inoculation technique

A sterile cottonwool swab was dipped into the suspension of the control inoculum, and was turned against the side of the container to remove excess fluid. Thereafter it was streaked on the top and bottom thirds of the plate leaving a central band uninoculated. (Fig. 1)

The test organism was streaked on the central band in the same manner as the control; and the discs were applied between the test and control organisms using a sharp needle.

Control organisms

The following control bacteria were used :-

for organisms isolated from urine : *Escherichia coli* NCTC 10418.

for bacteria from all other sites : *Staphylococcus aureus* NCTC 6571.

for *Pseudomonas species* : *Pseudomonas aeruginosa* NCTC 10662

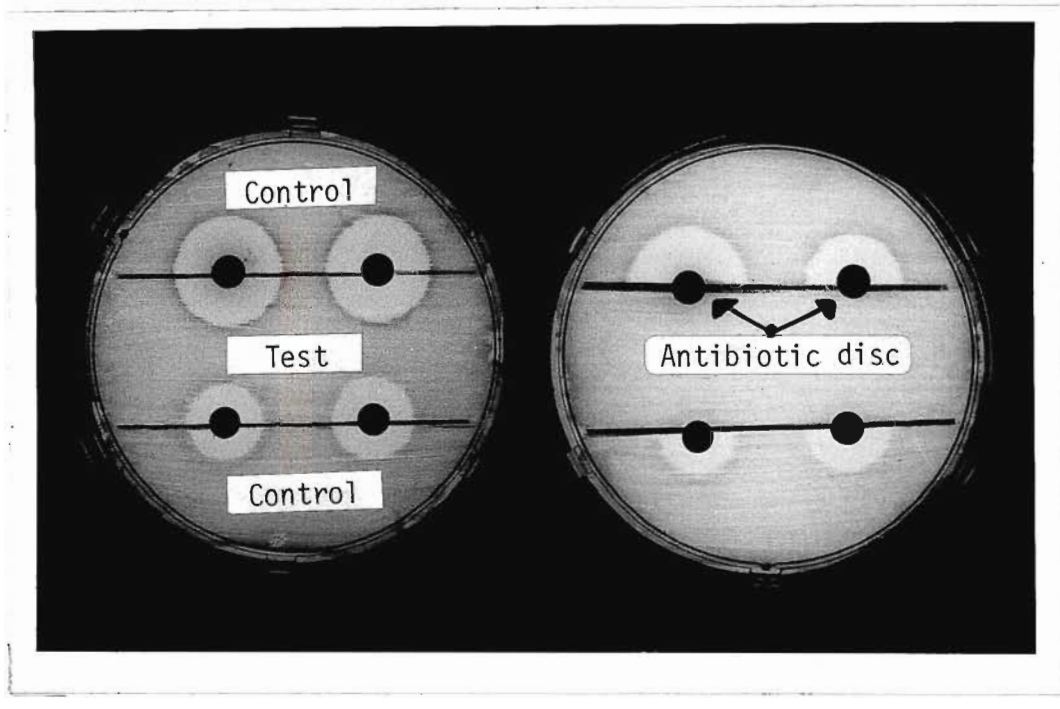

Fig. 1 Inoculation of Plates for the Stokes Method of Antibiotic Susceptibility Testing

Left Plate : Test organism is sensitive to the antibiotics
Right Plate : Test organism is resistant

Incubation

The plates were incubated at 37°C for 16-20 hours.

Antibiotic discs

Storage

Single discs were stored with a dessicant at 4°C in sealed containers clearly marked with the expiry date. They were allowed to come up to room temperature before the containers were opened. Discs were not used after the expiry date.

Choice of discs

The following range of antibiotic discs were used :-

(i) Gentamicin 10 µg per disc (Scherage (Pty) Ltd)

(ii) Tobramycin 10 µg per disc (Eli Lilly & Company)

(iii) Kanamycin 30 µg per disc (Bristol Laboratories)

(iv) Streptomycin 10 µg per disc (Glaxo Allenburys (Pty) Ltd.

(v) Carbenicillin 100 µg per disc (Beecham Laboratories)

(vi) Polymyxin B 300 units per disc (Mast Laboratories)

(vii) Amikacin 30 µg per disc (Bristol Laboratories)

(viii) Sisomicin 10 µg per disc (Bayer Pharmaceuticals)

Reading zones of inhibition

The density of the control and test inocula were comparable and within acceptable limits, i.e., semiconfluent. Tests with incorrect inocula were rejected and repeated.

Zone diameters were measured to the nearest millimetre with calipers.

Interpretation of the test

Sensitive : zone diameter wider than, equal to or not more than 6 mm smaller than the control.

Intermediate : zone more than 12 mm in diameter, but smaller than the control zone by more than 6 mm.

Resistant : zone diameter 12 mm or less.

Notes :

(i) For swarming Proteus species, the point of abrupt diminution of growth was taken as the zone edge, and swarming within the zone was disregarded.

(ii) Polymyxin : because Polymyxin B antibiotic diffuses slowly into the agar, zones were small and special criteria of interpretation was required, namely :

Sensitive : zone diameter wider than, equal to or not more than 6 mm smaller than the control.

Resistant : zone diameter more than 6 mm smaller than the control.

2.5.3 Determination of Minimum Inhibitory Concentrations

Minimum inhibitory concentrations (MIC's) were determined using an agar plate dilution technique.(148).

Medium

Mueller Hinton Agar (Oxoid) was used. The 85mm round petri plate takes 20 mls of agar. The amount of agar needed was based on this figure. Nineteen mls of agar was distributed in screw-capped bottles and maintained in a liquid state at 48°C to 50°C before the antibiotic solutions were added.

Antibiotic powders

Antibiotic powders suitable for susceptibility testing were obtained from the following companies :-

Antibiotic	Company	Potency
1. Gentamicin	Scherag (Pty) Ltd	546 µg/mg
2. Kanamycin	Bristol Laboratories	795 µg/mg
3. Tobramycin	Eli Lilly & Company	942 µg/mg
4. Amikacin	Bristol Laboratories	888 µg/mg
5. Sisomicin	Bayer Pharmaceuticals	591 µg/mg
6. Streptomycin	Glaxo Allenburys (Pty) Ltd	745-I.U./mg
7. Carbenicillin	Beecham Laboratories	810 µg/mg
8. Polymyxin B	Wellcome (Pty) Ltd	6493 I.U./mg

The antibiotic powders were stored under desiccation at -20°C.

Preparation of stock solution of antibiotics and serial dilutions (Table 2.1)

Example : Gentamicin Potency : 546 µg/mg

Concentration required : 40,000 µg/ml

Purity of gentamicin powder : 546 µg/mg

Correct for purity of powder : 40,000 ÷ 546

= 73,260 µg/ml

= 73,26 mg/ml

= 732,60 mg/10 ml

= 0,732 g/10 ml

= 7,32 g/100 ml

∴ Weigh 7,32 g antibiotic powder to 100 mls sterile distilled water. This is the 100 mls stock solution.

The stock solutions were distributed into 3 mls screw-capped bottles, sealed tightly and stored at -20°C. The bottles were removed from the freezer as needed and used within one day. Any unused, thawed stock solution was discarded.

Stock solutions were prepared in a similar manner for all other antibiotics used, making the necessary adjustment for purity.

Preparation of Plates

One ml of the diluted antibiotic was added to nineteen mls of the melted agar held at 48°C in one of the screw-capped bottles. The contents were mixed well by gently inverting the bottle several times and the agar was poured into the plates immediately. This process was repeated for each concentration of each antibiotic. A control plate without antibiotic was included in each run. The agar was allowed to solidify at room temperature. The plates were used on the same day.

Preparation of inoculum

The organisms to be tested were inoculated onto nutrient agar plates and incubated at 37°C for 16 - 20 hours. Portions of 4 - 5 discrete colonies

Table 2.1. A System for Preparing Dilutions for the Agar Dilution Method

Directions for preparing dilutions									*Final conc. µg per ml
1 ml	40000 µg/ml (stock solution)	+	Nil mls sterile distilled water				=	40000 µg/ml	2000
2 vols	40000 µg/ml	+	2 vol	"	"	"	=	20000 µg/ml	1000
1 vol	40000 µg/ml	+	3 vol	"	"	"	=	10000 µg/ml	500
1 vol	40000 µg/ml	+	7 vol	"	"	"	=	5000 µg/ml	250
2 vols	5000 µg/ml	+	2 vol	"	"	"	=	2500 µg/ml	125
1 vol	5000 µg/ml	+	3 vol	"	"	"	=	1250 µg/ml	62,5
1 vol	5000 µg/ml	+	7 vol	"	"	"	=	625 µg/ml	31,25
2 vols	625 µg/ml	+	2 vol	"	"	"	=	312,5 µg/ml	15,625
1 vol	625 µg/ml	+	3 vol	"	"	"	=	156,25 µg/ml	7,812
1 vol	625 µg/ml	+	7 vol	"	"	"	=	78,125 µg/ml	3,906
	etc							etc	etc

* The final concentration is based on the addition of 1 part of diluted antibiotic to 19 parts of melted agar.

were then inoculated into 4 mls of Tryptic soy broth (Difco) and incubated for 1 - 2 hours at 37°C. The turbidity of actively growing broth culture was then adjusted with Tryptic soy broth so as to obtain a turbidity visually comparable to that of a turbidity standard prepared by adding 0.5 ml of 0.048M $BaCl_2$ (1.175% [wt/vol] $BaCl_2 .2H_2O$) to 99.5 ml of 0.36 N H_2SO_4 (1%, vol/vol). This is half the density of a McFarland No. 1 standard and is often referred to as a McFarland 0.5 standard.

A 1:10 dilution was then prepared in Mueller-Hinton broth (Oxoid) for inoculation on the agar plates containing antibiotics. Inoculation of media was made within 30 minutes of adjusting the inoculum.

Inoculation of medium

The surface of the plates containing the dilutions of antibiotics and the control plates containing no antibiotic were allowed to dry, and the inoculum was applied as a spot with a multipoint inoculator (Denley Instruments, Sussex, England), which delivers 0.001 ml volumes. In each case about 10^4 CFU were delivered to a spot. (Fig. 2)

The plates containing the lowest concentration of antimicrobic were seeded first. Control plates were seeded first and last. Swarming by Proteus was prevented by pressing a glass cylinder (Raschig ring; Scientific Glass Apparatus, Bloomfield, N.J.) into the agar surrounding the inoculum spot.

Incubation

Inoculated agar plates were allowed to stand undisturbed until the inoculum spots were completely absorbed and were then incubated at 37°C for 16 - 20 hours.

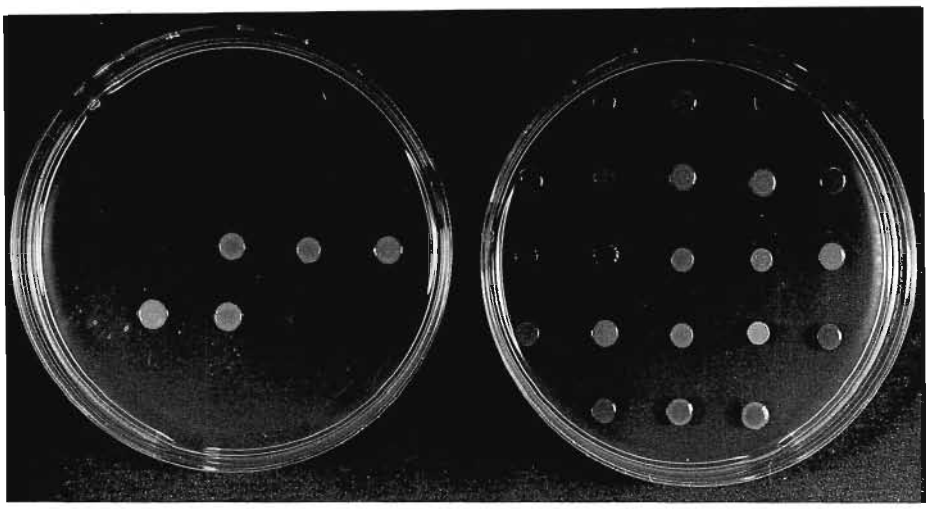

Fig. 2 Determination of M.I.C.

Right : Top row - control strains (*P. aeruginosa* NCTC 10662 and *E. coli* NCTC 10418)
Rows 2-5 - Test organisms - all organisms resistant to antibiotic concentration in the plate

Left : Increased antibiotic concentration in plate and growth of resistant organisms

Control strains

Staphylococcus aureus NCTC 6571, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662 of known behaviour were run with each batch of tests.

Results :

The MIC was considered to be the lowest antibiotic concentration at which complete inhibition occurred; a very fine, barely visible haze or a single colony was disregarded.

2.5.4. Pyocin Typing of *P. aeruginosa*

Two methods of pyocin typing have been successfully used :

(i) The Perpendicular Streak method of Gillies and Govan (151-153).

(ii) The Gratia Two-Layer technique (154) quoted by Peterson (155) Farmer *et al* (156) and Rampling *et al* (157,158).

In the preliminary studies, both the perpendicular streak and the two layer techniques were employed. Having tested fifty strains simultaneously, it was decided to use the two-layer technique in subsequent studies because difficulties were experienced in reading inhibition zones in the perpendicular streak method.

(i) The Perpendicular Streak Method

Culture media

(a) Tryptone soya agar (Oxoid) was prepared according to the manufacturer's instructions and five per cent defibrinated horse blood was incorporated.

(b) Nutrient broth No. 2 and nutrient agar (Oxoid) were also prepared according to the manufacturer's instructions.

Indicator strains

Eight indicator strains, numbers one to eight and five additional indicator strains, A - E were acquired from Dr R R Gillies in Edenburgh. These indicator strains were maintained on nutrient agar slopes, held at $4^{o}C$, and subcultures prepared every ten days.

Control Strains

P. aeruginosa strains of known pyocin type (type 1, type 16 and type 34) were also supplied by Dr Gillies. These strains were

used as controls in each batch of tests.

Method

(i) The strain of *P. aeruginosa* to be typed was subcultured onto nutrient agar plates to give single colonies, a day before the test.

(ii) Using a sterile cottonwool swab, colonies from the nutrient agar plate were streaked diametrically across the surface of tryptone soya blood agar (T S B A) to give an inoculum width of approximately one cm.

(iii) After incubation for 14 - 18 hours at 32°C, the growth from the T S B A was removed with a microscope slide which was dipped in chloroform.

(iv) The remaining viable growth was killed by pouring approximately 3 mls chloroform into the lid of the petri dish and replacing the medium-containing portion for 15 minutes.

(v) The plate was then opened and traces of chloroform vapour eliminated by exposing the medium to the air for a few minutes.

(vi) Cultures of the eight indicator strains, grown in nutrient broth for 4 hours at 37°C, were streaked onto the medium by means of a loop inoculator at right angles to the line of the original inoculum, so that five streaks were on the left and three on the right, as shown in Fig. 3.

(vii) The plate was then incubated at 37°C for 18 hours.

(viii) The pyocin types of the strains under examination were recognized from the patterns of inhibition which they produced on the eight indicator strains. (Table 2.2 and Fig. 4).

(ix) The results were recorded as follows :

+ = inhibition

- = no inhibition

(ii) The Two-layer technique

Culture media

Nutrient broth No. 2 and nutrient agar (Oxoid) were prepared according to the manufacturer's instructions.

Indicator & control strains

These were the same as those used in the Perpendicular Streak method.

Stock solutions

Buffered saline

0.1M NaCL + 0.01M Tris pH 7.6

1M $MnCl_2$ ph 7.5

Method

(a) Preparation of pyocin extracts

(i) The strain of *P. aeruginosa* to be typed was inoculated into 2 mls of nutrient broth and incubated at $37^{o}C$ for approximately 16 hours.

(ii) The overnight culture was then inoculated into 10 mls

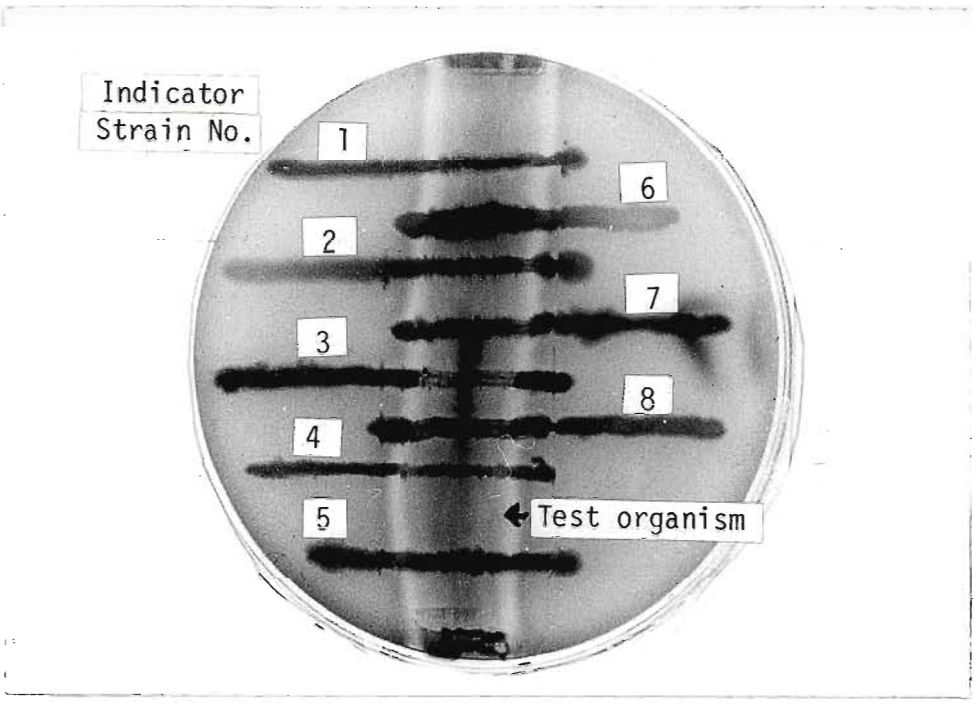

Fig. 3 The Perpendicular Streak Method of Pyocin Typing
Pyocin typing plate of a strain of *Pseudomonas aeruginosa* which is untypable, i.e. not producing detectable pyocine

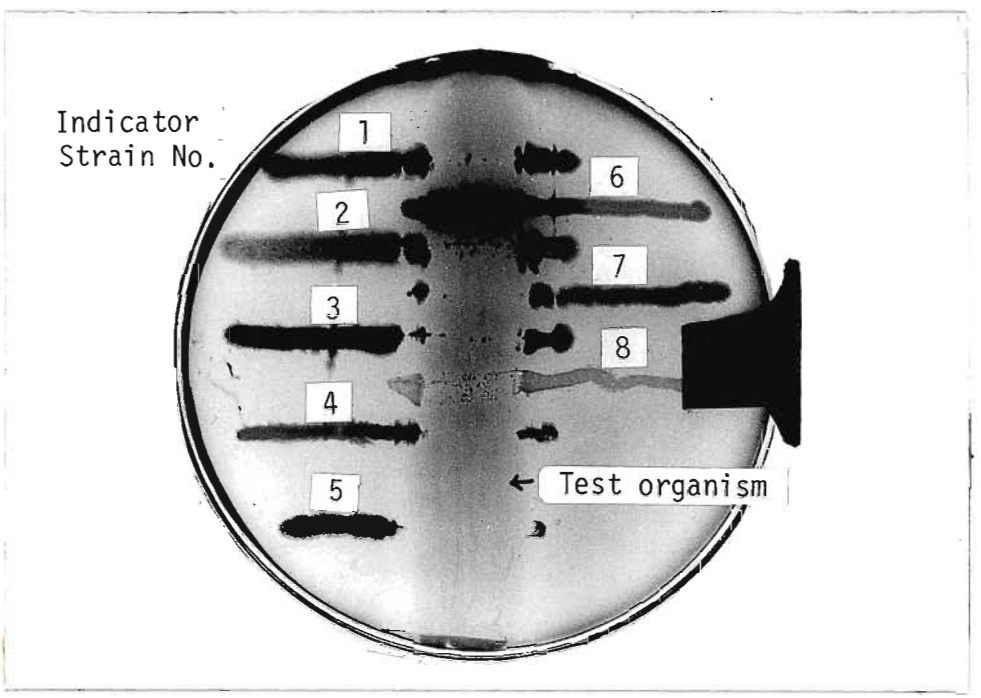

Fig. 4 Pyocin typing plate of a strain of *Pseudomonas aeruginosa* belonging to type 1, i.e. only indicator strain 6 (top right) remains uninhibited

of nutrient broth (all the sample was used) and incubated at 37°C for 2 hours.

(iii) Ten microlitres of Mitomycin C (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) was added to the suspension and incubated at 37°C for a further 2 hours.

(iv) Six mls of manganous chloride ($MnCl_2$) was then added to the suspension and the pH was checked and adjusted to 7,5 (should give a precipitate).

(v) The suspension was spun for 15 mins at 2000 rpm.

(vi) The supernatant was collected and saturated with 5 gms of ammonium sulphate.

(vii) The mixture was shaken to make sure that all the $(NH_4)_2SO_4$ had dissolved and recentrifuged as before.

(viii) The supernatant was discarded and the pellet was dissolved in 0,5 mls of buffered saline. (0.1M NaCL + 0.01M Tris at pH 7.6)

(ix) The sample was dialysed overnight against the same buffered saline, using the following procedure :-
A twenty cm length of dialysis tubing (Union Carbide Corporation, Chicago, Ill.) was used for each sample. A two litre glass beaker was filled with buffered saline and all of the tubing was immersed in this. The tubing was removed when it had turned opaque and was no longer brittle. A knot was made at the end of each piece of tubing, taking care not to puncture the tubing. Each of the tubes was filled with 0,5 ml sample. The

opposite ends of the tubing was sealed by a knot, and the tubing was placed back into the buffer. The longer end of the tubing was attached to the top of the beaker by means of masking tape. Each sample was labelled on the side of the beaker. The dialysis tubing was not allowed to dry out during operations. The beaker was then placed onto a magnetic stirrer for agitation. After 16-18 hours, the sample was removed from the dialysis tubing and poured into sterile petri dishes.

(x) The sample was then exposed to ultraviolet light by placing the petri dishes in the U.V. light chamber 40 cm away from the source, for 10 minutes, agitating the sample occasionally.

(xi) The sample (containing pyocin) was then placed into sterile bijou bottles.

(b) Preparation of overlay plates

(i) Each of the 8 indicator strains were cultured overnight at 37^{o}C in 10 mls nutrient broth. (This was done on the same day the test samples were prepared).

(ii) One drop of the indicator strain from the overnight culture was added to 5 mls of nutrient agar held molten at 50^{o}C.

(iii) This was mixed and poured as an overlay onto a nutrient agar plate and allowed to cool. One plate was prepared for each indicator strain.

(iv) The plates were allowed to dry for at least two hours. Each of the plates were labelled according to the indicator strain used.

(v) The prepared pyocins (test samples) were "spotted" onto the dried two-layered plates by means of a sterile pipette drawn to a fine bore. The pipette was held vertical and very close to the plate surface to prevent "bouncing" or "running" of the different pyocins into each other. Twenty pyocin preparations were tested on each plate. (Fig. 5)

(vi) The plates were allowed to dry on the bench before incubating overnight at $37^{o}C$.

(vii) The pyocin types of the strains under examination were recognised from the patterns of inhibition which they produced on the eight indicator strains. (Table 2.2)

(viii) The results were recorded as follows :-

+ = inhibition

- = no inhibition

N.B. (a) The technique for subdividing pyocin type - 1 strains is identical to that in the main typing procedures described except that five additional indicator strains are used to recognise eight subtype patterns. (Table 2.3)

(b) The sub-typing of pyocine type 1 was not done for this study.

(c) U/T = untypable strain, i.e. not producing detectable pyocin.

(d) U/C = unclassifiable strain, i.e. solitary strains giving patterns other than those in Gillie's classification.

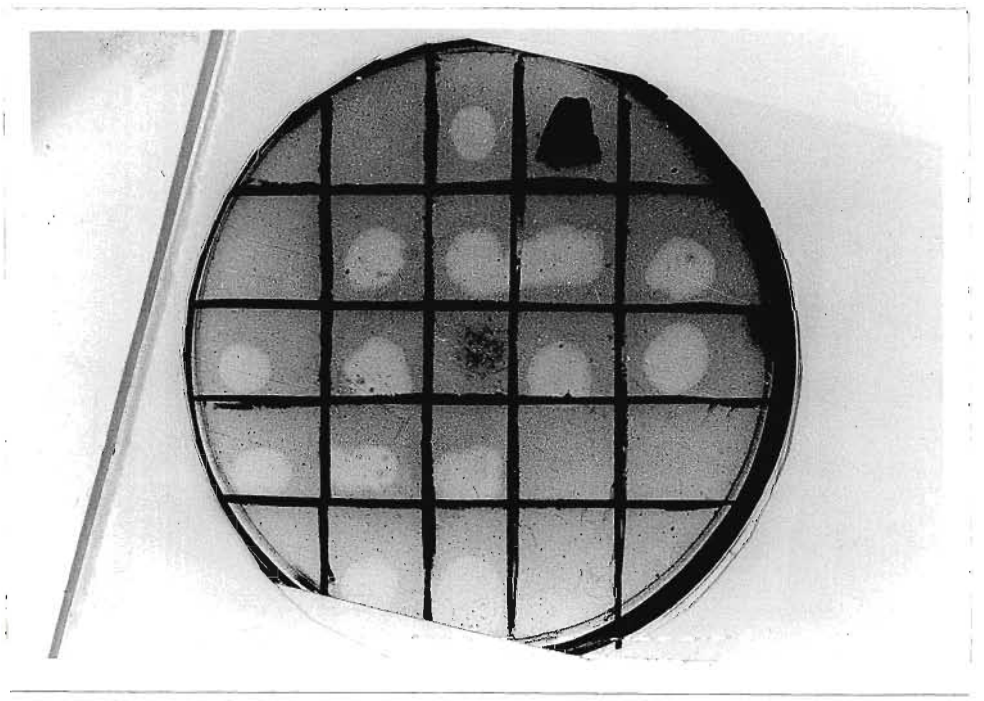

Fig. 5 Two-layer Technique of Pyocin Typing

Pyocin extracts were "spotted" onto a plate containing the indicator strain.

Table 2.2 Inhibition patterns of pyocin types of *Pseudomonas aeruginosa*

Pyocin type	Inhibition of indicator strain no.							
	1	2	3	4	5	6	7	8
1	+	+	+	+	+	-	+	+
2	-	+	-	-	-	-	-	-
3	+	+	+	-	+	-	+	-
4	+	+	+	+	+	-	-	+
5	-	-	-	-	+	-	-	-
6	+	+	+	+	+	-	+	-
7	+	+	+	-	-	-	+	+
8	-	+	+	+	-	-	+	-
9	-	-	-	-	+	-	+	-
10	+	+	+	+	+	+	+	+
11	+	+	+	-	-	-	+	-
12	+	+	-	+	+	-	-	+
13	-	-	-	+	-	-	-	+
14	-	-	+	-	+	-	+	-
15	-	+	-	-	+	-	+	-
16	+	-	+	+	-	-	+	+
17	-	-	+	-	-	-	+	-
18	+	-	+	+	+	-	+	+
19	-	-	+	+	-	-	+	-
20	-	-	-	-	+	+	-	-
21	-	+	-	+	+	-	-	-
22	+	+	+	-	+	+	+	-
23	+	-	-	-	+	-	+	-
24	-	-	+	+	+	-	+	+
25	+	-	+	-	-	-	+	-
26	+	-	-	-	-	-	+	-
27	+	-	+	-	+	-	+	-
28	-	-	-	+	-	-	+	-
29	-	+	-	-	+	-	-	-
30	-	+	+	-	-	-	-	-
31	-	-	-	-	-	-	+	-

continued...

Pyocin type	Inhibition of indicator strain no.							
	1	2	3	4	5	6	7	8
32	-	-	-	+	+	-	-	+
33	+	+	+	+	+	+	+	-
34	-	-	-	-	-	-	-	+
35	+	+	-	-	+	-	+	-
36	-	+	-	+	-	-	-	+
37	-	+	+	+	+	-	+	-
38	-	+	+	-	-	-	+	-
39	-	+	+	+	-	-	+	+
40	+	+	-	-	+	-	-	-
41	-	+	+	-	+	-	+	-
42	-	-	+	-	-	-	+	+
43	-	+	+	+	+	-	+	+
44	+	+	+	-	+	-	-	-
45	+	+	+	-	+	-	+	+
46	+	+	+	+	-	-	+	-
47	-	-	+	-	-	+	+	-
48	+	+	-	-	+	+	+	-
49	-	-	+	-	+	-	-	-
50	-	-	+	-	-	-	-	-
51	+	+	+	+	-	-	-	+
52	+	+	-	-	+	+	-	-
53	-	+	-	+	+	-	-	+
54	-	+	+	+	-	-	-	-
55	+	-	-	-	-	-	-	-
56	+	-	-	-	+	-	-	-
57	-	+	+	+	-	-	-	+
58	+	+	+	-	-	-	-	-
59	+	-	+	-	-	-	-	-
60	+	+	-	-	-	-	+	-
61	+	-	-	+	-	-	-	+
62	+	+	+	-	-	+	+	-
63	+	-	+	+	+	-	+	-
64	-	-	+	-	-	-	-	+
65	-	-	+	+	-	-	-	+

continued...

Pyocin type	Inhibition of indicator strain no.							
	1	2	3	4	5	6	7	8
66	-	+	+	-	+	-	-	-
67	-	-	-	-	-	+	+	-
68	-	-	-	+	-	-	-	-
69	-	+	+	-	-	+	+	-
70	-	+	-	+	-	-	-	-
71	-	+	-	+	-	-	+	-
72	-	+	-	-	+	+	-	-
73	-	+	-	-	-	+	-	-
74	-	+	+	-	-	-	+	+
75	+	-	+	+	-	+	+	-
76	+	+	-	+	+	+	+	-
77	-	+	+	-	-	-	-	+
78	-	+	-	+	-	+	+	+
79	-	-	-	+	+	+	+	+
80	-	+	-	+	-	-	+	+
81	-	+	-	-	+	-	-	+
82	-	+	-	-	+	-	+	+
83	-	-	-	+	+	-	+	+
84	-	+	-	-	-	-	+	-
85	-	+	-	-	-	-	+	+
86	-	+	+	-	-	+	-	-
87	-	+	+	+	-	+	-	+
88	+	-	-	-	+	-	-	+
89	-	+	+	+	-	+	-	-
90	-	-	+	+	-	-	-	-
91	-	-	+	+	-	+	-	-
92	-	-	+	+	+	-	+	-
93	+	-	-	+	-	-	-	-
94	+	-	-	+	+	-	-	+
95	+	-	+	-	-	+	+	-
96	+	-	+	+	-	-	-	-
97	+	-	+	+	+	-	-	+
98	+	-	+	+	-	-	+	-

continued....

Pyocin type	Inhibition of indicator strain no.							
	1	2	3	4	5	6	7	8
99	+	-	+	+	+	+	+	+
100	+	+	-	-	-	+	+	-
101	+	+	-	-	+	-	-	+
102	-	-	-	+	-	+	+	-
103	-	-	-	+	+	+	-	+
104	-	-	-	+	-	-	+	+
105	+	+	+	+	-	-	+	+

+ = Inhibition; - = no inhibition

Table 2.3 Patterns of inhibition produced by the 8 subtypes of pyocin type 1 of *Pseudomonas aeruginosa*

Pyocin subtype of type-1 producer strain*	Inhibition of indicator strain				
	A	B	C	D	E
a	+	+	+	+	+
b	-	+	+	+	+
c	-	-	+	+	+
d	+	-	+	+	+
e	-	+	+	-	+
f	-	-	-	-	-
g	-	-	+	-	+
h	-	+	-	+	+

*Strains in these subtypes are designated as "type 1a", "type 1b", etc.

2.5.5 Phage Typing of *P. aeruginosa*.

Phage typing techniques were generally those of Adams (159).

Media :

Nutrient broth : This was a modification of the broth of Coetzee and Sacks (160) and was made up as follows :

Difco nutrient broth powder	16 g
NaCl	10 g
Oxoid Lab-lemco broth powder	16 g
Difco tryptone broth powder	52 g

The above was dissolved in 2 litres distilled water and steamed for 45 minutes. Two ml of 7.5% $CaCl_2$ was added and adjusted to pH 7.4 with N-NaOH before autoclaving at 121°C for 15 minutes.

Phage agar : This was a modification of the nutrient agar of Adams (159) and was prepared as follows :

Difco agar	22 g
Difco bacto tryptone powder	26 g
NaCl	16 g
Glucose	3 g

Two litres distilled water was added and the medium autoclaved at 121°C for 15 minutes.

Phage agar top-layer : This was prepared as follows :

Difco agar	12 g
Difco bacto tryptone powder	20 g
NaCl	16 g
Glucose	6 g

These constituents were added to 2 litres distilled water, shaken

well and steamed for 45 minutes. The medium was bottled in 50 ml quantity and autoclaved at 121°C for 15 minutes. It was steamed for 1 hour before use.

Phage typing set

The following set of twenty-one phages with their host propagating bacterial strains were supplied by The National Collection of Type Cultures (NCTC), Colindale, London.

One ampoule (dried cultures and dried phage preparations) of each: 7, 16, 21, 24, 31, 44, 68, 73, F7, F8, F10, 109, 119X, 352, 1214, M4, M6, COL.21, COL.11, COL.18, 188/1.

The phages were labelled e.g. Ps.Ph.16 (Pseudomonas phage 16) and Ps.P.S.16 (Pseudomonas propagating strain 16).

Culture and phage

(a) High titre phage lysates were prepared directly from the freeze-dried stock material.

(b) The dried propagating strains were first suspended in one ml of nutrient broth and incubated overnight at 37°C.

(c) The following day, each of the 21 strains were streaked onto nutrient agar plates (for single colonies) and incubated overnight at 37°C.

(d) Single colonies from the nutrient agar plates were subcultured onto nutrient agar slants which were incubated overnight at 37°C.

(e) The slants were appropriately labelled with the strain number and held at 4°C for future use.

(f) The phages were reconstituted in one ml of nutrient broth from freeze dried material.

Propagation of phages

Phage lysates were prepared by the agar-overlay method of Swanstrom and Adams (214).

Materials : (all equipment must be sterile)

(a) Phage agar, in petri dishes (20 mls in 85 mm plates)

(b) Phage agar top layer in tubes, 2.5 ml

(c) Nutrient broth in tubes, 5 and 15 ml

Method :

(i) A tube of 5 ml nutrient broth, was inoculated with each propagating strain and incubated overnight at $37^{o}C$.

(ii) 0,5 ml of a 1:5 dilution of the overnight broth culture (3×10^{8} cells/ml) was added to 2.5 ml phage agar top layer held molten at $45^{o}C$.

(iii) This was overlaid on a phage agar plate. The plates were marked with the propagating strain number.

(iv) The semi-solid agar was evenly distributed by slowly rotating the dish.

(v) The plates were allowed to dry for 45 minutes at room temperature.

(vi) One drop of the standard phage was added to the surface of the dried plate of its homologous propagating strain.

(vii) The plates were incubated at $37^{o}C$.

(viii) The area showing lysis after overnight incubation was cut out and placed in 15 mls nutrient broth.

(ix) The broth was vigorously shaken by hand to break up agar pieces, and incubated at 37°C for 6 hours.

(x) The suspension was then centrifuged at 5,000 rpm for 30 minutes.

(xi) The supernatant was transferred to a sterile glass bottle and shaken with 2 mls chloroform.

(xii) The phage lysates were labelled according to their parent strains and stored at 4°C.

(xiii) Before use samples of phage suspensions were tested for sterility by spotting a few drops on MacConkey agar. Lysates were always sterile.

Assay of phage preparations

Determination of titre of phage stock solutions were made, using the techniques of Adams(159).

Materials :

(a) Phage agar, in petri dishes (20 mls in 85 mm plates). One plate is required for each of the 21 phages.

(b) Phage agar top layer in tubes, 2.5 ml.

(c) Nutrient broth in tubes, 0,9 and 5 mls.

Method :

(i) A tube of nutrient broth, 5 ml, was inoculated with each propagating strain and incubated overnight at 37°C.

(ii) 0,5 ml overnight broth culture of the propagating strain was mixed in small test tubes with 2,5 ml phage agar top layer at 45°C.

(iii) The mixture was overlaid on a phage agar plate. The plates were marked with the propagating strain number.

(iv) The semi-solid agar was evenly distributed by slowly rotating the dish.

(v) The plates were allowed to dry for 45 minutes at room temperature.

(vi) Dilution tubes were prepared by adding 0,9 mls of nutrient broth to a series of test tubes (12 tubes per phage).

(vii) Each tube was labelled with the phage number and appropriate dilution factor.

(viii) Serial 10 fold dilutions of the stock phage solutions were made up to a final dilution of 10^{-12}. Once the phages were diluted, the tubes were kept in the refrigerator when not in use. Separate pipettes were used for each dilution step.

(ix) By means of a one ml syringe, starting with the highest dilution and proceeding to the lowest, one drop of each dilution was placed in its proper place on the dried phage agar plate.

(x) The drops were allowed to absorb before inverting the plates.

(xi) The plates were incubated at $37^{o}C$ overnight.

(xii) The plates were removed and the degree of lysis at each dilution of phage was recorded as follows:

Reaction score	Plaques
++	more than 50 plaques; semi-confluent lysis; confluent lysis; corresponds to Routine Test Dilution (RTD).
+	20 - 50 plaques
±	less than 20 plaques

N.B.

(a) The titre of a phage solution is the number of plaque forming units per millilitre of stock solution.

(b) The RTD is that dilution of the phage suspension which gives semiconfluent lysis (more than 50 plaques) corresponding to the zone covered by the drop applied.

(c) Titres of $10^{11} - 10^{12}$ phage particles per ml., corresponding to a RTD of $10^{-4} - 10^{-5}$, were obtained by this method.

Determination of lytic spectrum

(a) This was carried out to detect any possible mutations or modifications which might have occurred during propagation.

(b) The lytic spectrum was determined by testing the phage against its propagating strain and a set of standard test strains, (Table 2.4).

(c) Determining the lytic spectrum of a phage consisted of finding out for each bacterial strain which phage dilution in 10-log steps ($^1/10$, $^1/100$, $^1/1000$, etc.) gave a ++ reaction. This was compared to the dilution which corresponded to RTD, i.e. gave a ++ reaction in the homologous host-propagating strain.

The degree of difference was scored as follows :

5 = maximum titre (i.e. on homologous propagating strain)

4 = 10^{-1} - 10^{-2} of titre on propagating strain

3 = 10^{-3} - 10^{-4} " " " " "

2 = 10^{-5} - 10^{-6} " " " " "

1 = very weak lysis

- = no reaction

(d) The standard lytic spectra of the phages are given in Table 2.4.

(e) The lytic spectrum of the standard phage preparations were performed exactly the same way as the newly made phage stock.

(f) The lytic spectra determination of the standard phage and the new phage preparation were carried out on the same day using the same batch of medium and the same broth cultures of the test strains.

(g) The two phage preparations (standard and stock) showed similar lytic spectrum. This made the newly prepared stock phage preparations acceptable for typing.

N.B. (i) High titre phages may inhibit many of the strains when used undiluted, but titration fails to reveal plaques. The inhibition in some cases may simulate confluent lysis but generally appears as a thinning of the growth in the drop area. Such reactions are entered as 0 on Table 2.4

(ii) In general the appearance of a grade 3,4 or 5 reaction where none should exist, or the complete absence of such a reaction, where one should exist, is an indication for rejection of a batch of phage. Variations of grade 4 to 5, 3 to 4 etc., and loss or gain of grade 1 and 2 reactions are permissible.

Table 2.4 Lytic Spectra of *Ps. aeruginosa* typing phages

Test strains	Phages																				
	7	16	21	24	31	44	68	73	F7	F8	F10	109	119X	352	1214	M4	M6	Col.11	Col.18	Col.21	188/1
7	5	-	0	-	0	0	0	0	-	0	-	0	4	-	0	4	-	4	-	0	-
16	0	5	0	-	-	-	5	-	-	0	2	0	0	-	0	-	-	-	-	-	-
21	3	0	5	-	-	2	5	-	4	0	-	5	5	-	0	-	0	-	-	0	5
24	-	-	0	5	-	5	4	-	0	3	-	5	-	5	5	4	-	4	-	-	5
31	0	0	4	-	5	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	4
44	-	-	5	-	-	5	0	-	5	0	-	0	4	-	4	-	-	-	-	-	4
68	0	-	-	-	-	0	5	-	0	4	-	5	0	0/3	5	-	-	-	-	-	-
73	0	3	0	-	-	0	5	5	5	0	-	-	5	0	2	0	4	5	-	0	-
F7	-	-	-	-	-	-	5	-	5	-	-	-	-	-	-	-	0	-	-	-	-
F8	1	5	0	5	-	5	5	2	0	5	-	5	5	3	5	4	4	5	-	0	-
F10	-	4	0	-	-	0	0	-	-	0	.5	0	0	-	-	-	-	-	-	-	-
109	-	0	-	3	-	-	-	-	-	0	-	5	-	3	4	-	-	-	0	0	0
119X	5	0	5	5	-	-	5	0	-	0	-	3	5	-	0	5	0	5	4	0	5
352	5	3	-	-	5	4	5	-	0	4	-	5	0	5	4	-	0	5	-	-	5
1214	-	2	5	1	-	2	5	2	-	3	2	3	2	1	5	-	-	-	1	3	-
M4	5	5	5	-	0/4	5	5	4	2	5	3	5	5	3	5	5	-	5	-	0	4
M6	-	1	5	2	-	1	5	1	0	-	2	1	4	2	4	3	5	-	-	3	0
Col.21	4	0/3	5	-	-	5	5	-	-	4	-	4	4	-	4	-	3	-	-	5	-

Notes: 1. Phages propagated on strains not included in the test set are also tested on their propagating strains, on which the reaction is by definition "5"

2. The notations 0/3 and 0/4 are used for reactions which are variable and which may appear as inhibition reactions on one occasion and as true lytic reactions on another.

Typing procedure

Materials :

(a) Phage agar in petri dishes (20 mls in 85 mm plates). 21 plates were used for typing each test organism.

(b) Phage agar top layer in tubes, 2.5 ml.

(c) Nutrient broth in tubes, 0,9 mls.

Method :

(i) 0,5 ml overnight broth culture of the test strain was mixed in small test tubes with 2,5 ml phage agar top layer at 45°C.

(ii) The mixture was poured as an overlay on the top of each phage agar plate. The plates were marked with the test strain number and the phage number. A total of 21 plates were prepared in this manner for typing each test strain.

(iii) The semi-solid agar was evenly distributed by slowly rotating the dish.

(iv) The plates were allowed to dry for 45 minutes at room temperature.

(v) Dilutions of the various pahges were made from neat to 10^{-7} with nutrient broth. (described earlier).

(vi) One drop of neat and diluted (10^{-1} to 10^{-7}) phage was spotted onto the 21 plates poured for each test strain (Fig 6).

(vii) The drops were allowed to absorb before inverting the plates.

(viii) The plates were incubated at 37°C overnight.

(ix) The following day, the plates were removed and the degree of lysis at each dilution of phage was recorded as stated

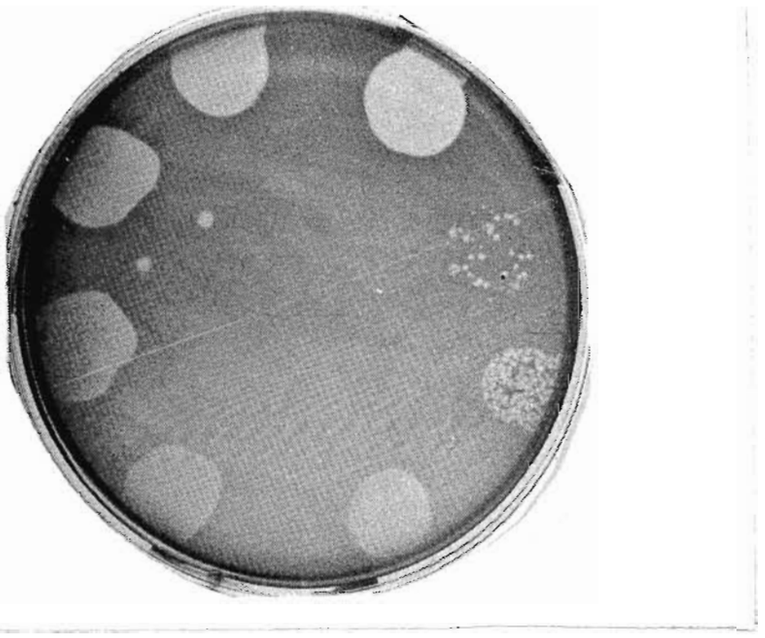

Fig. 6 Phage Typing Plate of *P. aeruginosa*

Test organism (0,5 ml overnight broth culture) was mixed with 2,5 ml phage agar top-layer and lawns poured on phage agar plates. Phage was spotted in dilutions on lawns after 45 minutes at room temperature.

previously.

N.B. The results of phage typing of a strain are reported in terms of those phages that produce strong lysis of the strain i.e., the phages that produce reactions of any degree from over 50 plaques to confluent lysis. This is the "phage pattern" of the strain, sometimes referred to as the "type".

2.5.6 Serological Typing of *P. aeruginosa*

O-serological typing

The antisera for serotyping were obtained from the Pasteur Institute, Paris, France. The basis of the Pasteur classification is that of Habs (290) , as modified and expanded by Veron (291). It corresponds to the proposed International Antigenic Typing Scheme (170,292).

Three polyvalent and 13 individual sera were provided in 2 ml vials.

A (types 1, 3, 4, 6, 10),

B (types 2, 5, 7, 8) and

C (types 9, 11, 12, 13).

The sera were supplied diluted, ready for use by the slide agglutination test.

The slide agglutination test

(i) The *P. aeruginosa* strain to be typed was grown on Trypticase soya agar (Difco) with 5% horse blood; incubated at 37^{o}C for 18-24 hours.

(ii) Four separate drops of saline were placed on a glass slab which was marked.

(iii) Part of a single colony of the culture to be typed was emulsified with a loop in each drop of saline to give smooth, fairly dense suspensions.

(iv) To one suspension, as a control, was added one drop of saline and to the other three suspensions were added one drop of pooled antisera, A, B and C.

(v) The suspensions were mixed by means of applicator sticks.

(vi) The glass slab was gently rocked and observed for agglutination which appeared within minutes.

(vii) The agglutination was characterised by a fine and regular bacterial clumping which was easily recognised. (Fig. 7).

N.B. (a) If the organism, agglutinated with one of the three pooled sera, then monovalent antisera were used to serotype the organism.

(b) If the organism failed to agglutinate with any sera, then the organism was labelled "non-agglutinable".

(c) If the orgnism agglutinated in the control, the strain was labelled "autoagglutinable".

(d) Some of the strains agglutinated with more than one monovalent antisera. These are shown in the results.

(e) The "non-agglutinable" and "autoagglutinable" strains are referred to as non-typable (NT) in the results.

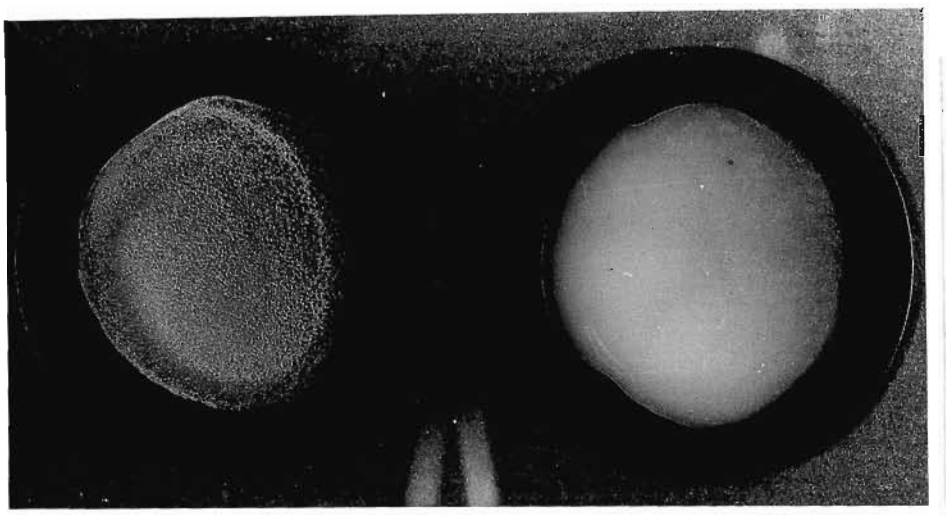

Fig. 7 Serological Typing of *P. aeruginosa*

Left : Positive agglutination

Right : Negative - no agglutination

RESULTS

3.1 INTRODUCTION

A total of 130 patients harbouring gentamicin-resistant gram-negative bacilli (GRGNB) were investigated. In addition 310 staff members associated with these patients were screened for carriage and transmission of GRGNB.

Environmental surveillance yielded 620 isolates of GRGNB whilst a total of 1083 GRGNB were obtained from clinical specimens.

The results are discussed under the following main headings :-

3.2 Wards Investigated

3.2.1 Intensive Care Unit (I C U) (Tables 3.1-3.5)

3.2.2 Respiratory Unit (R U) (Tables 3.6-3.10)

3.2.3 Paediatric Wards (P W) (Tables 3.11 - 3.15)

3.2.4 Surgical Wards (S W) (Tables 3.16-3.20)

3.2.5 Paediatric surgery and Burns Ward (PSB) (Tables 3.21-3.25)

3.2.6 Nursery (N) (Tables 3.26-3.30)

3.3 Identification and antibiotic susceptibility patterns of GRGNB isolates (Table 3.31) and cumulative MIC's of antimicrobial agents tested. (Tables 3.32.1-3.32.5)

3.4 Summary of the results obtained on patients, inanimate environment and staff members.

3.4.1 Distribution of 954 clinical isolates of GRGNB from all patients investigated. (Table 3.33)

3.4.2 Distribution of 620 isolates of GRGNB from all inanimate environments studied. (Table 3.34)

3.4.3 Distribution of clinical isolates of GRGNB from all staff members investigated. (Table 3.35)

3.5 Antibiotic usage of gentamicin and amikacin at K E H (Table 3.36).

A brief summary follows the presentation of results for isolates from the wards and environment.

3.2.1 INTENSIVE CARE UNIT (I C U)

Patients and Inanimate Environment

A total of 30 patients harbouring gentamicin-resistant gram negative bacilli were investigated over a 180 day period. The results are summarised in tables 3.1 - 3.3

Table 3.1 Antibiotic Susceptibility Pattern of GRGNB Isolates from Patients and Inanimate Environment

Organism	K E H Strain	G	T	K	St	C	P	A	S	Sero Type	Pyocin Type	Phage Type	Total (%)
P. aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	95(42,4)
P. aeruginosa	2	R	R	R	R	R	S	R	R	6/10	17	352	18 (8,0)
P. aeruginosa	3	R	R	R	R	R	S	R	R	11	U/C	109	18 (8,0)
P. aeruginosa	4	R	R	R	R	R	S	I	R	NT	U/T	21	17 (7,6)
P. aeruginosa	5	R	R	R	R	S	S	S	R	NT	21	31/68	9 (4,0)
P. cepacia		R	R	R	R	R	R	R	R	-	-	-	8 (3,6)
K. pneumoniae		R	R	R	R	R	S	S	R	-	-	-	43(19,2)
P. stuartii		R	R	R	R	R	R	S	R	-	-	-	16 (7,1)
TOTAL													224(100)

KEY : The five *Pseudomonas aeruginosa* strain listed i.e. 11,1,F7., 6/10,17,352,11,U/C,109;NT,U/T, 21 and NT,21,31/68 will be referred to as *P. aeruginosa* K E H strain 1-5 respectively.

G = GENTAMICIN
T = TOBRAMYCIN
K = KANAMYCIN
St= STREPTOMYCIN
C = CARBENICILLIN
P = POLYMYXIN B
A = AMIKACIN
S = SISOMICIN

R = RESISTANT, I = INTERMEDIATE and S = SENSITIVE

Figures in parentheses indicate % . These abbreviations will be used throughout this thesis.

Of the five different strains of *P. aeruginosa* isolated from this ward, K E H strain 1 was the commonest isolate, followed in decreasing order of frequency by K E H strain 2 (8%) and 3 (8%), K E H strain 4 (7,6%) and K E H strain 5 (4,0%). *K. pneumoniae* accounted for 19,2% of the isolates and *P. cepacia* and *P. stuartii* for 3.6% and 7,1% respectively.

All the isolates with the exception of *P. cepacia* and *P. stuartii* were sensitive to polymyxin B and 66% of all isolates were sensitive to both carbenicillin and amikacin. The isolates were generally resistant to most of the other antibiotics tested.

Table 3.2 Different Strains of GRGNB Isolated over a 180 Day Study Period

Organism	Days on which Isolated
P. aeruginosa K E H strain 1	6, 10, 12, 20, 22, 24, 25, 30, 31, 32, 33, 38, 40, 49, 50, 60, 70, 78, 80, 85, 112, 120, 125, 130, 135, 140, 145, 155, 165, 170, 175.
P. aeruginosa K E H strain 2	33, 45, 92, 170, 175, 180.
P. aeruginosa K E H strain 3	95, 100, 125, 130.
P. aeruginosa K E H strain 4	130, 135, 140, 145, 150, 154, 155, 172, 176, 180.
P. aeruginosa K E H strain 5	1, 4, 8, 10.
P. cepacia	30, 36, 45, 49, 53, 95, 100, 150, 154, 155.
K. pneumoniae	1, 4, 6, 8, 10, 20, 22, 24, 25, 33, 45, 49, 85, 92, 95, 100, 125, 130, 150, 154, 155, 175, 180.
P. sutartii	1, 4, 8, 10, 22, 24, 33, 45, 49, 60, 150, 154, 155, 176, 180.

P. aeruginosa K E H strain 3 and 5 were isolated for only a short period in contrast to *P. aeruginosa* K E H strain 1 and 2 and *K.pneumoniae* and *P. stuartii* which were found to persist throughout the period of study.

Table 3.3 Distribution of 224 GRGNB Isolates from Intensive Care Unit

Site	*P.aeruginosa* K E H strain 1	2	3	4	5	P.cepacia	K.pneumo	P.stuartii	Total (%)
Wound	11	4	1	4	2	1	4	-	27(18,1)
Stool	1	1	-	-	1	-	7	9	19(12,8)
Urine	11	1	-	-	1	-	2	1	16(10,7)
Groin & Axilla	10	-	2	2	-	-	2	-	16(10,7)
Endotracheal (aspirate)	3	-	2	2	2	2	2	-	13(8,7)
Blood	2	-	3	2	-	-	2	-	9(6,1)
Hand	1	-	2	-	-	-	5	-	8(5,4)
Umbilicus	3	1	-	-	-	-	2	-	6(4,0)
Trachea (Swab)	2	4	-	-	-	-	-	-	6(4,0)
Heart Blood (p.m.)	2	-	1	2	-	-	-	1	6(4,0)
Ear	5	-	-	-	-	-	-	-	5(3,4)
Sputum	3	-	-	-	-	-	2	-	5(3,4)
Eye	1	-	3	-	-	-	-	-	4(2,7)
Lung aspirate (p.m.)	1	-	-	1	-	-	-	1	3(2,0)
Throat	3	-	-	-	-	-	-	-	3(2,0)
Pleural aspirate	2	-	-	-	-	-	-	-	2(1,3)
Vagina	-	1	-	-	-	-	-	-	1(0,7)
Sub total	61 (40,9)	12 (8,1)	14 (9,4)	13 (8,7)	6 (4,0)	3 (2,0)	28 (18,8)	12 (8,1)	149(100)
*Inanimate environment	34 (45,3)	6 (8)	4 (5,3)	4 (5,3)	3 (4)	5 (6,7)	15 (20,0)	4 (5,3)	75 (100)
TOTAL	95 (42,4)	18 (8,0)	18 (8,0)	17 (7,6)	9 (4,0)	8 (3,6)	43 (19,2)	16 (7,1)	224 (100)

* This includes respiratory equipments, floors, walls, bed sheets, mattressses, sink surfaces and sink traps, water taps, urinals, bed-pans, baths, etc.

The common sites that yielded GRGNB were wound (18,1%), stool (12,8%), urine (10,7%) and skin which together accounted for more than 50% of the total isolates.

A total of 75 GRGNB were isolated from the immediate environment of these patients.

STAFF

A total of 50 staff members were screened for carriage and transmission of GRGNB. The results are summarised in tables 3.4 and 3.5

Table 3.4 Distribution of GRGNB in the Ward Staff

Staff - No.	*Hands		Rectal	Throat	Nasal	Staff** Clothing
	Before	After				
Nurses 25	Nil	6(24)	2(8,0)	1(4,0)	Nil	3(12,0)
Doctors 10	Nil	2(20)	Nil	Nil	Nil	2(20,0)
Cleaners 15	1(6,6)	4(26,6)	2(13,3)	1(6,6)	1(6,6)	3(20,0)

KEY :

*Before and two hours after commencement of duty.

** Uniforms, white-coats, gowns, aprons, and overshoes.

Of the staff members investigated, 6,6% of cleaners harboured GRGNB on their hands before commencement of duty and more than 20% of doctors, nurses and cleaners were positive for GRGNB two hours after commencement of duty.

Twenty per cent of doctors and cleaners and 12% of nurses harboured GRGNB on their clothing.

Table 3.5 Antibiotic Susceptibility Pattern of GRGNB Isolates from Staff Members

Organism	KEH strain	G	T	K	St	C	P	A	S	Sero type	Pyocin type	Phage type	Total (%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	13(46.4)
P.aeruginosa	4	R	R	R	R	R	S	I	R	NT	U/T	21	2(7,1)
P.aeruginosa	5	R	R	R	R	S	S	S	R	NT	21	31/68	4(14,3)
K.pneumoniae		R	R	R	R	R	S	S	R	-	-	-	8(28,6)
P.stuartii		R	R	R	R	R	R	S	R	-	-	-	1(3,6)
TOTAL													28(100)

The antibiotic susceptibility pattern of GRGNB isolates and the distribution of type strains were similar to those isolated from the patients and enviornment (c.f. table 3.1)

3.2.2. RESPIRATORY UNIT (R U)

Patients and Inanimate Environment

A total of 30 patients harbouring gentamicin-resistant gram negative bacilli were investigated over a 180 day period. The results are summarised in tables 3.6 - 3.8

Table 3.6 Antibiotic Susceptibility Pattern of GRGNB Isolates from Patients and Inanimate Environment

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total (%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	113 (30,8)
P.aeruginosa	6	R	R	R	R	S	S	S	R	3,10	9	16	55 (15,0)
P.aeruginosa	7	R	R	R	R	I	S	S	R	NT	35	31	24 (6,5)
P.aeruginosa	8	R	R	R	R	R	S	I	R	NT	U/C	M6	17 (4,6)
A.anitratus		R	R	R	R	R	S	S	R	-	-	-	79 (21,5)
K.pneumoniae		R	R	R	R	R	S	S	R	-	-	-	59 (16,1)
F.meningosepticum		R	R	R	R	R	R	R	R	-	-	-	20 (5,4)
TOTAL													367 (100)

KEY :

The four *Pseudomonas aeruginosa* strain listed i.e. 11,1,F7; 3/10,9,16; NT,35,31 and NT,U/C,M6 will be referred to as *P.aeruginosa* K E H strain 1, 6, 7, 8 respectively.

Of the four different strains of *P. aeruginosa* isolated from this ward, K E H strain 1 was the commonest isolate, followed in decreasing order of frequency by K E H strain 6 (15,0%), K E H strain 7 (6,5%) and K E H strain 8 (4,6%). *A. anitratus* accounted for 21,5% of the isolates and *K. pneumoniae* and *Flavobacterium meningosepticum* for 16,1% and 5,4%, respectively.

All the isolates with the exception of *F. meningosepticum* were sensitive to polymyxin B. Forty-six per cent of all isolates were sensitive to carbenicillin and 90% to amikacin. The isolates were generally resistant to most of the other antibiotics tested.

Table 3.7 Different Strains of GRGNB Isolated over a 180 Day Study Period

Organism	Days on which Isolated
P. aeruginosa KEH strain 1	1, 5, 10, 12, 25, 28, 30, 35, 50, 58, 78, 84, 93, 95, 98, 130, 134 135, 137, 138, 140, 142, 145, 150, 152, 160, 180.
P. aeruginosa KEH strain 6	93, 95, 98, 110, 115, 120, 123, 125, 126, 130, 133, 140, 142, 144, 150.
P. aeruginosa KEH strain 7	1, 3, 5, 6, 15, 20, 23, 70, 75, 89, 92, 100, 105, 110, 140, 144, 170, 174.
P. aeruginosa KEH strain 8	60, 68, 70, 75, 78, 84, 86, 90, 142, 145.
A. anitratus	1, 5, 93, 95, 98, 123, 126, 130, 133, 135, 137, 140, 170, 174, 175, 180.
K. pneumoniae	10, 12, 60, 68, 70, 75, 100, 105, 110, 112, 114, 123, 126, 130, 133, 134, 138, 140, 160, 168, 180.
Flavo.meningosepticum	10, 12, 60, 68, 70, 75, 100, 105, 110, 112, 114, 123, 126, 130, 133, 134, 138, 140, 160, 175.

P. aeruginosa K E H strain 6 and 8 were isolated for only a short period in contrast to K E H strain 1 and 7 and *A. anitratus*, *K. pneumoniae* and *F. meningosepticum* which were found to persist throughout the period of study.

Table 3.8 Distribution of 367 GRGNB Isolates from Respiratory Unit

Site	P.aeruginosa K E H strain 1	6	7	8	A. anitratus	K. pneumo	Flavo. meningo	Total (%)
Endotracheal(aspirate)	10	6	2	-	10	6	1	35(13,2)
Stool	8	4	1	2	8	10	-	33(12,4)
Trachea (swab)	6	4	4	-	6	4	4	28(10,5)
Hand	6	6	3	3	6	4	-	28(10,5)
Urine	7	2	2	2	4	6	-	23(8,6)
Sputum	6	2	2	1	4	4	3	22(8,3)
Throat	4	4	2	2	2	2	2	18(6,8)
Groin & Axilla	4	3	2	2	4	-	-	15(5,6)
Wound	3	-	1	1	6	1	1	13(4,9)
Ear	5	-	2	1	2	-	-	10(3,8)
Vagina	4	3	-	-	2	-	-	9(3,4)
Umbilicus	3	3	1	-	1	-	-	8(3,0)
Blood	5	1	-	-	-	1	-	7(2,6)
Heart Blood (P.M.)	5	1	-	-	-	1	-	7(2,6)
Lung aspirate (P.M.)	5	1	-	-	-	1	-	7(2,6)
Pleural aspirate	1	1	-	-	-	-	-	2(0,8)
Eye	1	-	-	-	-	-	-	1(0,4)
Sub Total	83 (31,2)	41 (15,4)	22 (8,3)	14 (5,3)	55 (20,7)	40 (15,0)	11 (4,1)	266(100)
*Inanimate environment	30 (29,7)	14 (13,9)	2 (2,0)	3 (3,0)	24 (23,8)	19 (18,8)	9 (8,9)	101 (100)
Total	113 (30,8)	55 (15,0)	24 (6,5)	17 (4,6)	79 (21,5)	59 (16,1)	20 (5,4)	367 (100)

* This includes respiratory equipment, floors, walls, bed sheets, mattresses, sink surfaces and sink traps, water taps, urinals, bed pans, medicines, food etc.

The common sites that yielded GRGNB were endotracheal aspirates (13,2%), stool (12,4%), trachea (10,5%) and skin which together accounted for more than 50% of the total isolates.

A total of 101 GRGNB were isolated from the immediate environment of these patients.

STAFF

A total of 60 staff members were screened for carriage and transmission of GRGNB.

The results are summarised in tables 3.9 and 4.0

Table 3.9 Distribution of GRGNB in the Ward Staff

Staff - No.	Hands		Rectal	Throat	Nasal	Staff Clothing
	Before	After				
Nurses 30	Nil	7(23,3)	1(3,3)	1(3,3)	Nil	10(33,3)
Doctors 10	Nil	3(30,0)	2(20,0)	1(10,0)	Nil	1(10,0)
Cleaners 20	2(10,0)	6(30,0)	3(15,0)	2(10,0)	1(5,0)	6(30,0)

Of the staff members investigated, 10% of cleaners harboured GRGNB on their hands before commencement of duty and more than 20% of doctors, nurses and cleaners were positive for GRGNB two hours after commencement of duty.

Thirty-three per cent of nurses, 30% of cleaners and 10% of doctors, harboured GRGNB on their clothing.

Table 3.10 Antibiotic Susceptibility Pattern of GRGNB Isolates from Staff Members.

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total(%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	28(60,9)
P.aeruginosa	7	R	R	R	R	I	S	S	R	NT	35	31	2(4,3)
K.pneumoniae		R	R	R	R	R	S	S	R	-	-	-	10(21,7)
A.anitratus		R	R	R	R	R	S	S	R	-	-	-	4(8,7)
F.meningosepticum		R	R	R	R	R	R	R	R	-	-	-	2(4,3)
Total													46(100)

The antibiotic susceptibility pattern of GRGNB isolates and the distribution of type stains were similar to those isolated from the patients and environment (c.f. table 3.6)

3.2.3. PAEDIATRIC WARDS (P.W.)

Patients and Inanimate Environment

A total of 20 patients harbouring gentamicin-resistant gram negative bacilli were investigated over a 180 day period. The results are summarised in tables 3.11 - 3.13.

Table 3.11 Antibiotic Susceptibility Pattern of GRGNB Isolates from Patients and Inanimate Environment

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total (%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	46(20,3)
P.aeruginosa	7	R	R	R	R	I	S	S	R	NT	35	31	12 (5,3)
P.cepacia		R	R	R	R	R	R	R	R				23(10,1)
P.stutzeri		R	R	R	R	R	S	S	R				7 (3,1)
Flavo. meningosepticum		R	R	R	R	R	R	R	R				25(11,0)
E. coli		R	R	R	R	R	S	S	R				21 (9,3)
K.pneumoniae		R	R	R	R	R	S	S	R				18 (7,9)
K.aerogenes		R	R	R	R	R	S	I	R				17 (7,5)
P.morganii		R	R	R	R	R	R	S	R				16 (7,0)
E.cloacae		R	R	R	R	R	S	R	R				15 (6,6)
A.anitratus		R	R	R	R	R	S	S	R				15 (6,6)
C.freundii		R	R	R	R	R	S	S	R				12 (5,3)
Total													227(100)

KEY : The two *Pseudomonas aeruginosa* strain listed i.e. 11, 1, F7 and NT, 35, 31 will be referred to as *P.aeruginosa* K E H strain 1 and 7 respectively.

Of the pseudomonads isolated, *P. aeruginosa* K E H strain 1 accounted for 20,3%, *P. cepacia* (10,1%), *P. aeruginosa* K E H strain 7 (5,3%) and *P. stutzeri* (3,1%).

Of the enterobacteria, *Flavobacterium meningosepticum*, *E. coli* and *Klebsiella* were the most frequent isolates.

The isolates were generally resistant to most of the antibiotics tested with the exception of polymyxin B and amikacin.

Table 3.12 Different Strains of GRGNB Isolated over a 180 Day Study Period

Organism	Days on which Isolated
P.aeruginosa KEH strain 1	1, 4, 6, 20, 25, 60, 65, 95, 100, 108, 112, 130, 165, 170, 180
P.aeruginosa KEH strain 7	10, 15, 35, 40, 82, 90, 130, 140, 171, 175
P. stutzeri	35, 40, 43, 46, 50
P. cepacia	35, 40, 48, 50, 55, 60, 66, 72
F. meningosepticum	4, 20, 30, 35, 58, 84, 110, 140, 162, 180.
E. coli	1, 4, 6, 35, 40, 82, 90, 150, 160, 172, 175, 180.
K. pneumoniae	1, 4, 6, 10, 15, 30, 35, 70, 78, 47, 52, 60, 65, 70.
P. morganii	1, 4, 6, 115, 118, 130, 135.
E. cloacae	1, 4, 6, 10, 30.
A. anitratus	20, 25, 35, 40, 171, 175
C. freundii	20, 25.

With the exception of *P. aeruginosa* K E H strain 1, K E H strain 7, *E. coli* and *Flavo. meningosepticum*, the remaining organisms were isolated only for intermittent periods of the study.

Table 3.13 Distribution of 227 GRGNB Isolates from Paediatric Wards

Site	P.aerug KEH strain 1	P.aerug KEH strain 7	P cepacia	P stutzeri	Flavo meningo	E.coli	K pneumo	K aerogenes	P morganii	E cloacae	A anitratus	C freundii	Total(%)
Stool	-	2	6	1	4	2	4	2	3	2	1	4	31(20,9)
Urine	6	-	4	-	4	1	1	2	2	-	-	-	20(13,5)
Hand	-	2	-	2	2	3	1	2	1	2	2	-	17(11,5)
Vagina	4	-	3	-	3	3	-	2	-	1	-	-	16(10,8)
Wound	4	-	-	-	1	2	2	1	1	-	2	-	13 (8,8)
Ear	2	-	-	1	1	-	-	-	1	1	2	4	12 (8,1)
Groin & Axilla	5	2	-	-	-	2	-	-	-	-	-	-	9 (6,1)
Umbilicus	1	1	-	-	2	1	-	-	1	1	1	1	9 (6,1)
Sputum	-	1	-	-	2	1	3	2	-	-	-	-	9 (6,1)
Throat	3	-	-	1	2	-	-	-	-	-	2	-	8 (5,4)
Eye	1	-	-	-	-	1	-	-	-	-	-	-	2 (1,4)
Pleural Aspirate	-	-	-	-	-	-	-	-	-	-	1	-	1 (0,7)
Blood	-	-	-	-	-	-	-	-	1	-	-	-	1 (0,7)
Sub total	26 (17,6)	8 (5,4)	13 (8,8)	5 (3,4)	21 (14,2)	16 (10,8)	11 (7,4)	11 (7,4)	10 (6,8)	7 (4,7)	11 (7,4)	9 (6,1)	148(100)
*Inaminate Environment	20 (25,3)	4 (5,1)	10 (12,7)	2 (2,5)	4 (5,1)	5 (6,3)	7 (8,9)	6 (7,6)	6 (7,6)	8 (10,1)	4 (5,1)	3 (3,8)	79 (100)
Total	46 (20,3)	12 (5,3)	23 (10,1)	7 (3,1)	25 (11,0)	21 (9,3)	18 (7,9)	17 (7,5)	16 (7,0)	15 (6,6)	15 (6,6)	12 (5,3)	227 (100)

* This includes floors, walls, bed sheets, mattresses, sink surfaces and sink traps, water taps, urinals, bed-pans, foods, medicines, disinfectants, etc.

The common sites that yielded GRGNB were stool (20,9%), urine (13,5%) and skin which together accounted for more than 50% of the total isolates.

A total of 79 GRGNB were isolated from the immediate environment of these patients.

STAFF

A total of 60 staff members were screened for carriage and transmission of GRGNB.

The results are summarised in tables 3.14 and 3.15

Table 3.14 Distribution of GRGNB in the Ward Staff

Staff - No.	Hands		Rectal	Throat	Nasal	Staff Clothing
	Before	After				
Nurses 30	Nil	8(26,6)	2(6,6)	1(3,3)	Nil	10(33,3)
Doctors 10	Nil	1(10,0)	ND	ND	ND	1(10,0)
Cleaners 20	Nil	4(20,0)	1(5,0)	Nil	1(5,0)	2(10,0)

ND = not done

Of the staff members investigated, none of them harboured GRGNB on their hands before commencement of duty but 26,6% of nurses, 10% of doctors and 20% of cleaners were positive for GRGNB two hours after commencement of duty.

Thirty - three percent of nurses and 10% of doctors and cleaners harboured GRGNB on their clothing.

Table 3.15 Antibiotic Susceptibility Pattern of GRGNB Isolates from Staff Members

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total (%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	4(12,9)
P.aeruginosa	7	R	R	R	R	I	S	S	R	NT	35	31	2(6,5)
K.pneumoniae		R	R	R	R	R	S	S	R				8(25,8)
E. coli		R	R	R	R	R	S	S	R				6(19,4)
A.anitratus		R	R	R	R	R	S	S	R				5(16,1)
C.freundii		R	R	R	R	R	S	S	R				4(12,9)
E. cloacae		R	R	R	R	R	S	R	R				2(6,5)
Total													31(100)

The antibiotic susceptibility pattern of GRGNB isolates and the distribution of type strains were similar to those isolated from the patients and environment (c.f. table 3.11)

3.2.4 SURGICAL WARDS (SW)

Patients and Inanimate Environment

A total of 20 patients harbouring gentamicin-resistant gram negative bacilli were investigated over a 180 day period. The results are summarised in Tables 3.16-3.18.

Table 3.16 Antibiotic Susceptibility Pattern of GRGNB Isolates from Patients and Inanimate Environment

Organism	KEH strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total(%)
P.aeruginosa	9	R	R	R	R	R	S	S	R	6	19	24	78(27,6)
P.aeruginosa	2	R	R	R	R	R	S	R	R	6/10	17	352	22(7,8)
P.aeruginosa	10	R	R	R	R	I	S	S	R	9	5	1214	18(6,4)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	8(2,8)
P. rettgeri		R	R	R	R	R	R	S	R				50(17,7)
K.pneumoniae		R	R	R	R	R	S	S	R				41(14,5)
A.anitratus		R	R	R	R	R	S	S	R				38(13,4)
P.stuartii		R	R	R	R	R	R	S	R				28(9,9)
Total													283(100)

KEY : The four *Pseudomonas aeruginosa* strain listed i.e. 6, 19, 24., 6/10, 17, 352., 9, 5, 1214 and 11, 1 , F7 will be referred to as *P.aeruginosa* KEH strain 9, 2, 10 and 1 respectively.

Of the four different strains of *P. aeruginosa* isolated from this ward, K E H strain 9 was the commonest isolate, followed in decreasing order of frequency by KEH strain 2 (7,8%), KEH strain 10 (6,4%) and KEH strain 1 (2,8%). *Proteus rettgeri* accounted for 17,7% of isolates and *K. pneumoniae A. anitratus* and *P. stuartii* for 14,5%, 13,4% and 9.9% respectively.

All the isolates, with the exception of *P. rettgeri* and *P. stuartii* were sensitive to polymyxin B. Ninety-two per cent of all isolates were sensitive to amikacin and 2,8% to carbenicillin.

The isolates were generally resistant to most of the other antibiotics tested.

Table 3.17 Different Strains of GRGNB Isolated over a 180 Day Study Period

Organism	Days on which Isolated
P.aeruginosa KEH strain 9	1, 4, 8, 10, 14, 16, 25, 30, 35, 40, 70, 80, 85, 90, 95, 98, 115, 120, 142, 150, 171, 180,
P.aeruginosa KEH strain 2	25, 30, 65, 68, 75, 100.
P.aeruginosa KEH strain 10	65, 68, 80, 90, 100, 103, 107.
P.aeruginosa KEH strain 1	120, 125, 140, 150, 155, 170, 180.
P. rettgeri	115, 120, 142, 150, 160, 168, 172, 175, 180.
K.pneumoniae	10, 14, 16, 20, 30, 35, 40, 55, 60, 70, 80, 95, 98, 142, 150, 172, 180.
A. anitratus	1, 4, 8, 30, 35, 40, 45, 50, 55, 60, 90, 95, 110, 113, 120, 125, 140, 160, 178.
P. stuartii	10, 14, 16, 30, 35, 40, 70, 80, 103, 107, 142, 150, 160, 165, 172, 179.

P. aeruginosa KEH strain 1, 2, 10 and *P. rettgeri* were isolated for only a short period in contrast to *P. aeruginosa* K E H strain 9, *K. pneumoniae*, *A. anitratus* and *P. stuartii* which were found to persist throughout the period of study.

Table 3.18 Distribution of 283 GRGNB Isolates from Surgical Wards

Site	P.aeru.KEH strain No 9	2	10	1	P.rettgeri	K.pneumoniae	A.anitratus	P.stuartii	Total(%)
Wound	8	1	-	2	2	4	2	1	20(14,3)
Groin & Axilla	6	3	2	0	-	1	4	2	18(12,9)
Stool	4	2	-	1	3	2	1	2	15(10,7)
Umbilicus	4	1	2	0	4	1	2	1	15(10,7)
Ear	4	2	1	0	1	1	2	1	12(8,6)
Sputum	5	-	-	1	2	3	1	-	12(8,6)
Vagina	2	1	1	0	3	1	1	2	11(7,9)
Hand	4	1	-	-	1	1	2	2	11(7,9)
Urine	3	1	-	1	2	2	1	1	11(7,9)
Throat	3	-	-	-	2	2	2	1	10(7,1)
Eye	1	-	-	-	-	-	-	-	1(0,7)
Blood	1	-	-	-	-	-	-	-	1(0,7)
Heart Blood (PM)	1	-	-	-	-	-	-	-	1(0,7)
Lung aspirate (PM)	1	-	-	-	-	-	-	-	1(0,7)
Pleural aspirate	1	-	-	-	-	-	-	-	1(0,7)
Sub total	48	12	6	5	20	18	18	13	140(100)
	(34,3)	(8,6)	(4,3)	(3,6)	(14,3)	(12,9)	(12,9)	(9,3)	
*inanimate environment	30	10	12	3	30	23	20	15	143
	(21,0)	(7,0)	(8,4)	(2,1)	(21,0)	(16,1)	(14,0)	(10,5)	(100)
Total	78	22	18	8	50	41	38	28	283
	(27,6)	(7,8)	(6,4)	(2,8)	(17,7)	(14,5)	(13,4)	(9,9)	(100)

* This includes floors, walls, bed sheets, mattresses, sink surfaces and sink traps, water taps, urinals, bed pans, food, medicines, disinfectants, etc.

The common sites that yielded GRGNB were wound (14,3%), stool (10,7%) and skin which together accounted for more than 50% of the total isolates.

A total of 143 GRGNB were isolated from the immediate environment of these patients.

STAFF

A total of 60 staff members were screened for carriage and transmission of GRGNB.

The results are summarised in tables 3.19 and 3.20.

Table 3.19 Distribution of GRGNB in the Ward Staff

Staff-No	Hands		Rectal	Throat	Nasal	Staff Clothing
	Before	After				
Nurses 35	Nil	7(20,0)	3(8,5)	1(2,8)	Nil	7(20,0)
Doctors 10	Nil	1(10,0)	Nil	Nil	Nil	3(30,0)
Cleaners 15	1(6,6)	4(26,6)	4(26,6)	1(6,6)	1(6,6)	5(33,0)

Of the staff members investigated, 6,6% of cleaners harboured GRGNB on their hands before commencement of duty and 20% nurses, 10% doctors and 26,6% cleaners were positive for GRGNB two hours after commencement of duty.

Twenty per cent of the nurses, 30% doctors and 33% cleaners harboured GRGNB on their clothing.

Table 3.20 Antibiotic Susceptibility Pattern of GRGNB Isolates from Staff Members

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total (%)
P.aeruginosa	9	R	R	R	R	R	S	S	R	6	19	24	11(28,9)
P.aeruginosa	2	R	R	R	R	S	S	S	R	6/10	17	352	9(23,7)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	8(21,1)
A.anitratus		R	R	R	R	R	S	S	R	-	-	-	6(15,8)
P.stuartii		R	R	R	R	R	R	S	R	-	-	-	4(10,5)
TOTAL													38(100)

The antibiotic susceptibility pattern of GRGNB isolates and the distribution of type strains were similar to those isolated from the patients and environment (c.f. Table 3.16)

3.2.5 PAEDIATRIC SURGERY AND BURNS WARD (PSB)

Patients and Inanimate Environment

A total of 20 patients harbouring gentamicin-resistant gram negative bacilli were investigated over a 180 day period. The results are summarised in Tables 3.21-3.23.

Table 3.21 Antibiotic Susceptibility Pattern of GRGNB Isolates from Patients and Inanimate Environment

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total (%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	64(19,5)
P.aeruginosa	2	R	R	R	R	R	S	R	R	6/10	17	352	35(10,6)
P.aeruginosa	11	R	R	R	R	S	S	S	R	NT	35	7/44	7 (2,1)
P.cepacia		R	R	R	R	R	R	R	R				11 (3,3)
P.stutzeri		R	R	R	R	R	S	S	R				6 (1,8)
E. coli		R	R	R	R	R	S	S	R				39(11,9)
A.anitratus		R	R	R	R	R	S	S	R				37(11,2)
Flavo.meningo		R	R	R	R	R	R	R	R				25 (7,6)
P.rettgeri		R	R	R	R	R	R	S	R				22 (6,7)
E.aerogenes		R	R	R	R	R	S	S	R				21 (6,4)
P.morganii		R	R	R	R	S	R	S	R				20 (6,1)
K.aerogenes		R	R	R	R	R	S	I	S				18 (5,5)
A.faecalis		R	R	R	R	R	S	R	R				12 (3,6)
P.mirabilis		R	R	R	R	R	R	S	R				12 (3,6)
TOTAL													329 (100)

KEY: The three *Pseudomonas aeruginosa* strain listed i.e. 11,1,F7,6/10,17,352,NT,35,7/44 are referred to as *P.aeruginosa* K E H strain 1, 2 and 11 respectively.

Of the pseudomonads, *P. aeruginosa* K E H strain 1, 2 and 11 accounted for 32,2% of the isolates, *P. cepacia* 3,3% and *P. stutzeri* 1,8%.

Of the enterobacteria, *E. coli*, *A. anitratus* and *Flavobacterium meningosepticum* represented the most frequent isolates.

The isolates were generally resistant to most of the antibiotics tested with the exception of polymyxin B and amikacin.

Table 3.22 Different Strains of GRGNB Isolated over a 180 Day Study Period

Organism	Days on which Isolated
P. aeruginosa KEH strain 1	1, 3, 5, 8, 10, 12, 14, 16, 20, 21, 23, 24, 25 28, 30, 34, 39, 40, 45, 50, 55, 60, 65, 70, 100, 110, 120, 125.
P. aeruginosa KEH strain 2	12, 14, 16, 30, 35, 40, 80, 85, 120, 125, 140, 145.
P. aeruginosa KEH strain 11	140, 145.
P. cepacia	5, 8, 10, 45, 50, 55, 90, 95, 150, 155.
P. stutzeri	5, 8, 10, 38, 40, 43, 140, 145.
E. coli	1, 3, 5, 8, 10, 12, 18, 24, 25, 28, 60, 65, 70, 90, 95.
A. anitratus	80, 85, 150, 155.
Flavo.meningosepticum	15, 18, 20, 30, 34, 39, 40, 43, 75, 80, 150.
P. rettgeri	38, 40, 43.
E. aerogenes	75, 80.
P. morganii	15, 18, 20, 30, 34, 39, 60, 65, 70, 150.
K. aerogenes	1, 3, 5, 8, 12, 14, 16, 75, 80.
A. faecalis	1, 3, 5, 8, 24, 25, 28, 45, 50, 55.
P. mirabilis	90, 95, 130, 135.

All the gentamicin resistant pseudomonads and enterobacteria were isolated for only a short period in contrast to *P. aeruginosa* KEH strain 1 which was found to persist throughout the period of study.

Table 3.23 Distribution of 329 GRGNB Isolates from Paediatric Surgery and Burns Ward

Site	P.aeru KEH strain 1	2	11	P.Cepacia	P.stutzeri	E.coli	A.anitratus	Flavo. meningo	P.Rettgeri	E.aerogenes	P.morganii	K.aerogenes	A. faecalis	P. mirabilis	Total (%)
Skin (burn)	10	5	2	2	2	4	4	3	3	2	3	4	2	3	49(23,3)
Stool	4	3	1	-	1	3	3	2	1	2	3	1	1	1	26(12,4)
Umbilicus	4	4	-	1	-	2	2	1	3	1	3	1	-	1	23(11,0)
Sputum	3	2	1	-	-	1	3	3	1	-	1	1	2	1	19 (9,0)
Hand	4	3	-	1	-	-	2	1	2	2	2	2	-	-	19 (9,0)
Urine	3	2	-	-	-	2	1	1	2	2	1	-	1	2	17 (8,1)
Groin & Axilla	5	3	-	-	-	3	2	-	1	-	-	2	-	-	16 (7,6)
Ear	4	1	-	-	-	1	1	1	1	1	1	2	-	-	13 (6,2)
Throat	2	2	-	-	-	1	1	2	1	1	-	1	-	1	12 (5,7)
Wound	1	-	-	-	-	1	2	1	1	-	-	-	-	-	6 (2,9)
Vagina	1	-	-	1	-	1	-	-	1	-	-	-	1	-	5 (2,4)
Blood	1	-	-	-	-	-	1	-	-	-	1	-	-	-	3 (1,4)
Heart Blood (PM)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (0,5)
Lung Aspirate (PM)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (0,5)
SUB TOTAL	44	25	4	5	3	19	22	15	17	11	15	14	7	9	210(100)
	(21,0)	(11,9)	(1,9)	(2,4)	(1,4)	(9,0)	(10,5)	(7,1)	(8,1)	(5,2)	(7,1)	(6,7)	(3,3)	(4,3)	
*Inanimate environment	20	10	3	6	3	20	15	10	5	10	5	4	5	3	119
	(16,8)	(8,4)	(2,5)	(5,0)	(2,5)	(16,8)	(12,6)	(8,4)	(4,2)	(8,4)	(4,2)	(3,4)	(4,2)	(2,5)	(100)
TOTAL	64	35	7	11	6	39	37	25	22	21	20	18	12	12	329
	(19,5)	(10,6)	(2,1)	(3,3)	(1,8)	(11,9)	(11.2)	(7,6)	(6,7)	(6,4)	(6,1)	(5,5)	(3,6)	(3,6)	(100)

* This includes floors, walls, bed sheets, mattresses, sink surfaces and sink traps, water taps, urinals, bed pans, foods, medicines, disinfectants etc.

The common sites that yielded GRGNB were skin including burn (39,9%), stool (12,4%) and sputum (9,0%) which together accounted for more than 50% of the total isolates.

A total of 119 GRGNB were isolated from the immediate environment of these patients.

STAFF

A total of 50 staff members were screened for carriage and transmission of GRGNB.

The results are summarised in tables 3.24 and 3.25.

Table 3.24 Distribution of GEGNB in the Ward Staff

Staff-No	Hands		Rectal	Throat	Nasal	Staff Clothing
	Before	After				
Nurses 25	Nil	6(24,0)	2(8,0)	2(8,0)	1(4,0)	10(40,0)
Doctors 10	Nil	1(10,0)	ND	Nil	Nil	3(30,0)
Cleaners 15	1(6,6)	6(40,0)	3(20,0)	2(13,3)	1(6,6)	6(40,0)

ND = NOT DONE

Of the staff members investigated, 6,6% of cleaners harboured GRGNB on their hands before commencement of duty and 10% doctors, 24% nurses and 40% cleaners were positive for GRGNB two hours after commencement of duty.

Forty per cent of the nurses and cleaners and 30% doctors harboured GRGNB on their clothing.

Table 3.25 Antibiotic Susceptibility Pattern of GRGNB Isolates from Staff Members

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total (%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	14(31,8)
P.aeruginosa	11	R	R	R	R	S	S	S	R	NT	35	7/44	5(11,4)
K.pneumoniae		R	R	R	R	R	S	S	R				8(18,2)
A.anitratus		R	R	R	R	R	S	S	R				5(11,4)
E.coli		R	R	R	R	R	S	S	R				4 (9,1)
A.faecalis		R	R	R	R	R	S	R	R				4 (9,1)
Flavo.meningo		R	R	R	R	R	R	R	R				4 (9,1)
TOTAL													44(100)

The antibiotic susceptibility pattern of GRGNB isolates and the distribution of type strains were similar to those isolated from the patients and environment (c.f. Table 3.21)

3.2.6 NURSERY

Patients, Boarder Mothers and Inanimate Environment

A total of 10 patients and 10 boarder mothers harbouring gentamicin-resistant gram-negative bacilli were investigated over a 180 day period. The results are summarised in tables 3.26 - 3.28

Table 3.26 Antibiotic Susceptibility Pattern of GRGNB Isolates from Patients, Board Mothers and Inanimate Evnironment

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	TOTAL(%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	21(32,8)
K.pneumoniae		R	R	R	R	R	S	S	R				29(45,3)
E. coli		R	R	R	R	R	S	S	R				14(21,9)
TOTAL													64(100)

KEY : The *Pseudomonas aeruginosa* strain listed i.e. 11, 1, F7 will be referred to as *P. aeruginosa* K E H strain 1.

K. pneumoniae was the commonest isolate from this ward. *P. aeruginosa* K E H strain 1 and *E. coli* accounted for 32,8% and 21,9% of the isolates respectively.

All the isolates were sensitive to both polymxin B and amikacin. Thirty-three per cent of all isolates were sensitive to carbenicillin. The isolates were generally resistant to most of the other antibiotics tested.

Table 3.27 Different Strains of GRGNB Isolated over a 180 Day Study Period

Organism	Days on which Isolated
P. aeruginosa KEH strain 1	1, 10, 30, 40, 48, 60, 100, 140, 163, 174, 180.
K. pneumoniae	1, 5, 20, 45, 60, 84, 100, 128, 145, 163, 174, 180.
E. coli	1, 3, 20, 24, 28.

E. coli was isolated for only a short period in contrast to *P. aeruginosa* strain 1 and *K. pneumoniae* which were found to persist throughout the period of study.

Table 3.28 Distribution of 64 GRGNB Isolates from Nursery

Site	P.aeruginosa KEH strain 1	K.pneumoniae	E.coli	TOTAL (%)
Stool	3	10	4	17 (41,5)
Mother's hands	4	6	3	13 (31,7)
Eye	3	1	1	5 (12,2)
Umbilicus	1	4	-	5 (12,2)
C S F	-	-	1	1 (2,4)
Sub total	11 (26,8)	21 (51,2)	9 (22,0)	41 (100)
* Inanimate environment	10 (43,5)	8 (34,8)	5 (21,7)	23 (100)
TOTAL	21 (32,8)	29 (45,3)	14 (21,9)	64 (100)

* This includes floors, walls, baby cots, napkins, sink surfaces and sink traps, water taps, baby feeds, medicines etc.

The common sites that yielded GRGNB were stool (41,5%) and mother's hands which together accounted for more than 70% of the total isolates.

A total of 23 GRGNB were isolated from the immediate environment of these patients.

STAFF

A total of 30 staff members were screened for carriage and transmission of GRGNB.

The results are summareised in tables 3.29 and 3.30.

Table 3.29 Distribution of GRGNB in the Ward Staff

Staff-No.	Hands		Rectal	Throat	Nasal	Staff Clothing
	Before	After				
Nurses 15	-	3(20,0)	1 (6,6)	Nil	1 (6,6)	4(26,6)
Doctors 5	-	1(20,0)	Nil	Nil	Nil	1(20,0)
Cleaners 10	1(10,0)	(2(20,0)	3(30,0)	1(10,0)	1(10,0)	3(30,0)

Of the staff members investigated, 10% of cleaners harboured GRGNB on their hands before commencement of duty and 20% doctors, nurses and cleaners were positive for GRGNB two hours after commencement of duty.

Twenty per cent of doctors, 26,6% of nurses and 30% of cleaners harboured GRGNB on their clothing.

Table 3.30 Antibiotic Susceptibility Pattern of GRGNB Isolates from Staff Members

Organism	K E H strain	G	T	K	ST	C	P	A	S	Sero type	Pyocin type	Phage type	Total (%)
P.aeruginosa	1	R	R	R	R	S	S	S	R	11	1	F7	10 (45,5)
K. pneumoniae		R	R	R	R	R	S	S	R				8 (36,4)
E. coli		R	R	R	R	R	S	S	R				4 (18,2)
TOTAL													22 (100)

The antibiotic susceptibility pattern of GRGNB isolates and the distribution of type strains were similar to those isolated from the patients, boarder mothers and environment (c.f. Table 3.26)

Table 3.31 Identification and Antibiotic Susceptibility Patterns of GRGNB Isolates from All Wards Investigated

Organism	K E H strain	Sero type	Pyocin type	Phage type	G	T	K	ST	C	P	A	S	Total No isolates (%)
P.aeruginosa	1	11	1	F7	R	R	R	R	S	S	S	R	424(24,9)
P.aeruginosa	9	6	19	24	R	R	R	R	R	S	S	R	89 (5,2)
P.aeruginosa	2	6,10	17	352	R	R	R	R	R	S	R	R	84 (4,9)
P.aeruginosa	6	3,10	9	16	R	R	R	R	S	S	S	R	55 (3,2)
P.aeruginosa	7	NT	35	31	R	R	R	R	I	S	S	R	40 (2,3)
P.aeruginosa	4	NT	U/T	21	R	R	R	R	R	S	I	R	19 (1,1)
P.aeruginosa	3	11	U/C	109	R	R	R	R	R	S	R	R	18 (1,1)
P.aeruginosa	10	9	5	1214	R	R	R	R	I	S	S	R	18 (1,1)
P.aeruginosa	8	NT	U/C	M6	R	R	R	R	R	S	I	R	17 (1,0)
P.aeruginosa	5	NT	21	31/68	R	R	R	R	S	S	S	R	13 (0,8)
P.aeruginosa	11	NT	35	7/44	R	R	R	R	S	S	S	R	12 (0,7)
P.cepacia	-	-	-	-	R	R	R	R	R	R	R	R	42 (2,5)
P.stutzeri	-	-	-	-	R	R	R	R	R	S	S	R	13 (0,8)
K.pneumoniae					R	R	R	R	R	S	S	R	232(13,6)
A.anitratus					R	R	R	R	R	S	S	R	189(11,1)
E. coli					R	R	R	R	R	S	S	R	88 (5,2)
Flavo.meningosepticum					R	R	R	R	R	R	R	R	76 (4,4)
P.rettgeri					R	R	R	R	R	R	S	R	72 (4,2)
P.stuartii					R	R	R	R	R	R	S	R	49 (2,9)
P.morganii					R	R	R	R	S	R	S	R	36 (2,1)
K.aerogenes					R	R	R	R	R	S	I	R	35 (2,1)
E.aerogenes					R	R	R	R	R	S	S	R	21 (1,2)
E.cloacae					R	R	R	R	R	S	R	R	17 (1,0)
C.freundii					R	R	R	R	R	S	S	R	16 (0,9)
A.faecalis					R	R	R	R	R	S	R	R	16 (0,9)
P.mirabilis					R	R	R	R	R	R	S	R	12 (0,7)
Total													1703(100)

Table 3.32.1 Cumulative Percentage of 844 GRGNB* Inhibited by Eight Antimicrobial Agents

	Drug Concentration (μg/ml or I.U./ml)									
Antibiotic	<3.9	7.8	15.6	31.3	62.5	125	250	500	1000	>2000
Gentamicin	-	-	4,5	15,2	37,1	50,9	72,7	81,8	92,9	100
Tobramycin	-	-	4,7	27,3	41,5	41,5	75,1	88,2	97,6	100
Kanamycin	-	-	-	-	4,7	14,2	35,5	64,0	89,7	100
Streptomycin	-	-	-	-	-	-	2,5	15,4	39,1	100
Carbenicillin	-	-	8,5	29,9	66,6	72,9	81,5	87,2	97,4	100
Polymyxin B	95,0	-	-	-	-	-	-	-	-	100
Amikacin	14,2	59,2	78,7	82,9	100					
Sisomicin	-	-	-	-	8,8	20,1	42,2	57,1	79,9	100

* *Pseudomonas species* (789 *P. aeruginosa*, 42 *P.cepacia* and 13 *P.stutzeri*)

Table 3.32.2 Cumulative Percentage of 267 GRGNB* Inhibited by Eight Antimicrobial Agents

	Drug concentration (µg/ml or I.U./ml)						
Antibiotic	<3.9	7.8	15.6	31.3	62.5	125	>250
Gentamicin	-	11,2	33,3	74,5	83,9	91,4	100
Tobramycin	-	13,1	36,0	77,9	88,4	96,3	100
Kanamycin	-	-	-	13,1	28,1	74,9	100
Streptomycin	-	-	-	15,7	50,6	67,8	100
Carbenicillin	-	-	-	-	-	31,5	100
Polymyxin B	100						
Amikacin	41,2	74,9	86,9	99,6	100		
Sisomicin	-	-	-	3,7	48,7	73,4	100

**Klebsiella species* (232 *K. pneumoniae* and 35 *K. aerogenes*)

Table 3.32.3 Cumulative Percentage of 169 GRGNB* Inhibited by Eight Antimicrobial Agents

	Drug Concentration (μg/ml or I.U./ml)						
Antibiotic	<3.9	7.8	15.6	31.3	62.5	125	>250
Gentamicin	-	8,9	32,5	72,8	84,6	94,1	100
Tobramycin	-	12,4	37,3	78,7	92,9	96,4	100
Kanamycin	-	-	-	1,2	23,1	47,9	100
Streptomycin	-	-	-	0,6	14,2	21,9	100
Carbenicillin	-	-	5,3	16,6	21,3	49,7	100
Polymyxin B	-	-	-	-	-	-	100
Amikacin	85,8	100					
Sisomicin	-	-	7,1	29,0	53,3	83,4	100

* Proteus - Providence Group (72 *P. rettgeri*, 36 *P. morganii*, 12 *P. mirabilis* and 49 *P. stuartii*)

Table 3.32.4 Cumulative Percentage of 142 GRGNB* Inhibited by Eight Antimicrobial Agents

	Drug Concentration (µg/ml or I.U./ml						
Antibiotic	<3.9	7.8	15.6	31.3	62.5	125	>250
Gentamicin	-	4,2	14,8	57,0	94,4	97,9	100
Tobramycin	-	2,8	16,9	59,2	95,1	97,9	100
Kanamycin	-	-	-	25,4	58,5	93,0	100
Streptomycin	-	-	-	4,2	18,3	65,5	100
Carbenicillin	-	-	-	-	-	56,3	100
Polymyxin B	100						
Amikacin	68,3	79,6	88,0	94,4	100		
Sisomicin	-	-	7,0	36,6	63,4	84,5	100

* All other fermenters (88 *E.coli*, 21 *E. aerogenes*, 17 *E.cloacae* and 16 *C. freundii*)

Table 3.32.5 Cumulative Percentage of 281 GRGNB* Inhibited by Eight Antimicrobial Agents

	Drug Concentration (μg/ml or I.U./ml)						
Antibiotic	<3.9	7.8	15.6	31.3	62.5	125	>250
Gentamicin	-	2,8	24,2	50,9	82,9	90,0	100
Tobramycin	-	4,6	26,7	55,2	81,5	89,3	100
Kanamycin	-	-	-	12,5	32,4	64,4	100
Streptomycin	-	-	-	5,0	52,7	65,8	100
Carbenicillin	-	-	-	-	-	60,9	100
Polymyxin B	73	-	-	-	-	-	100
Amikacin	47,7	61,9	67,3	82,2	93,6	100	
Sisomicin	-	-	3,6	13,5	39,9	80,4	100

* All other non-fermenters (189 *A. anitratus*, 76 *Flavo.meningosepticum* and 16 *A. faecalis*)

Table 3.33 Distribution of 954 Clinical Isolates of GRGNB from all Patients Investigated

Site	ICU	RU	PW	SW	PSB	N	Total (%)
Stool	19	33	31	15	26	17	141(14,8)
Urine	16	23	20	11	17	-	87 (9,1)
Hand	8	28	17	11	19	-	83 (8,7)
Wound	27	13	13	20	6	-	79 (8,3)
Groin & axilla	16	15	9	18	16	-	74 (7,8)
Sputum	5	22	9	12	19	-	67 (7,0)
Umbilicus	6	8	9	15	23	5	66 (6,9)
Ear	5	10	12	12	13	-	52 (5,5)
Throat	3	18	8	10	12	-	51 (5,3)
Skin (burn)	-	-	-	-	49	-	49 (5,1)
Endotracheal (aspirate)	13	35	-	-	-	-	48 (5,0)
Vagina	1	9	16	11	5	-	42 (4,4)
Trachea (swab)	6	28	-	-	-	-	34 (3,6)
Blood	9	7	1	1	3	-	21 (2,2)
Heart blood (P.M.)	6	7	-	1	1	-	15 (1,6)
Eye	4	1	2	1	-	5	13 (1,4)
Mother's hands	-	-	-	-	-	13	13 (1,4)
Lung aspirate (P.M.)	3	7	-	1	1	-	12 (1,3)
Pleural aspirate	2	2	1	1	-	-	6 (0,6)
C S F	-	-	-	-	-	1	1 (0,1)
TOTAL	149	266	148	140	210	41	954(100)

Table 3.34 Distribution of 620 Isolates of GRGNB from all Inanimate Environments Studied

	ICU	RU	PW	SW	PSB	N	Total (%)
Sinks	12	16	15	20	19	5	87(14,0)
Clothes - staff	8	17	13	15	19	8	80(12,9)
Wound dressings	3	1	4	30	20	-	58 (9,4)
Clothes - patients	6	8	3	10	4	2	33 (5,3)
Water taps	4	6	4	10	5	2	31 (5,0)
Bed Sheets	4	4	3	5	10	1	27 (4,4)
Air - settle plates	10	6	2	6	3	-	27 (4,4)
Respiratory equipment	6	20	-	-	-	-	26 (4,2)
Ward sluice rooms	3	4	4	4	7	3	25 (4,0)
Bed pans	3	4	4	10	4	-	25 (4,0)
Urinals	3	3	4	8	5	-	23 (3,7)
Urinary catheters	3	-	6	8	4	-	21 (3,4)
Floors	3	4	4	3	4	1	19 (3,1)
Floor mops	2	3	4	8	1	1	19 (3,1)
Toilet seats	-	2	6	2	6	-	16 (2,6)
Soaps	4	3	2	1	4	1	15 (2,4)
Mattresses	1	2	3	2	6	-	14 (2,3)
Baths	-	2	4	2	5	-	13 (2,1)
Suction apparatus	-	-	-	4	2	4	10 (1,6)
Disinfectant solutions	2	4	1	1	1	-	9 (1,5)
Walls	2	2	1	2	1	-	8 (1,3)
Nail brushes	1	1	2	2	1	-	7 (1,1)
Ward kitchens	-	-	2	3	1	-	6 (1,0)
Food	-	1	1	-	2	2	6 (1,0)
Medicines	-	1	-	1	1	1	4 (0,6)
Nasopharyngeal catheters	1	-	-	-	3	-	4 (0,6)
Flower vase water	1	2	-	1	-	-	4 (0,6)
Resuscitation equipment	1	2	-	-	-	-	3 (0,5)
Total	83	118	92	158	138	31	620(100)

Table 3.35 Distribution of Clinical Isolates of GRGNB from All Staff Members Investigated

Site	No.*	ICU	RU	PW	SW	PSB	N	Total (%)
Hands - before	310	1	2	NIL	1	1	1	6
Hands - after		12	16	13	12	13	6	72 } 78(60,5)
Rectum	290	4	6	3	7	5	4	29(22,5)
Throat	300	2	4	1	2	4	1	14 (10,9)
Nose	300	1	1	1	1	2	2	8 (6,2)
Total		20	29	18	23	25	14	129 (100)

* No. of staff investigated

Table 3.36 Usage of Injectable Gentamicin and Amikacin at King Edward VIII Hospital for the Period 1979-1983

Year	Gentamicin(g) used	Amikacin(g) Used
1979	1318	*
1980	1568	*
1981	2259	258
1982	3096	469
1983	3700	545

* = No figures available

DISCUSSION

One of the objectives of this study was to determine the role of resistant pseudomonads and enterobacteria in hospital cross-infection. A study of the patients harbouring such organisms, their immediate environment and the staff responsible for their care is one way of providing such information. It is necessary also, to obtain isolates from as many individuals, representing each of these sources, as possible, in order that such a study may be statistically significant.

The use of modern epidemiological typing methods to elucidate nosocomial infections and the spread of communicable diseases is fully recognized today. Phage typing, serotyping, bacteriocin (gero) typing, biochemical typing, typing by antibiogram and R-plasmids are methods used either separately or together to obtain a more detailed level of differentiation within bacterial species. The history of the practical use of these methods runs parallel to the history of the development of phage typing as a supplementary and very necessary approach to epidemiological investigation.

The discussion that follows is divided firstly into considering the methodology used in this study. An attempt is then made to define the extent and nature of this bacterial resistance as well as the sources in case of hospital infections of such organisms.

4.1 Pyocin Typing

This is a method used to elucidate nosocomial infections caused by *Pseudomonas aeruginosa*. Pyocins are the bacteriocins of *P. aeruginosa*, i.e. antibiotic substances produced by strains of the species which have the characteristic property of being lethal only for other strains of *P. aeruginosa*.

Holloway (92) reported that pyocinogeny was common in strains of *P. aeruginosa* and suggested that pyocin production might prove a useful epidemiological marker of this species. Table 3.31 shows that 7 pyocin types were identified from a total of 789 *P. aeruginosa* strains investigated. *P. aeruginosa* pyocin type 1 was the commonest type and found in all the wards studied. This type was isolated from clinical specimens, inanimate environment and also from staff members. This pyocin type referred to as *P. aeruginosa* K E H strain 1 in this study, seems to be the "resident flora" of this hospital, since it was isolated throughout the study period (c.f. Tables 3.1-3.30).

Pyocin type 1 constituted 53,7% of the *P. aeruginosa* isolates. This was followed in decreasing frequency by pyocin type 19 (11,3%), type 17 (10,6%), type 9 (7,0%), type 35 (6,6%) and types 21 and 5 both of which made up less than 5%. Thirtyfive (4,4%) of the 789 strains tested were found to be unclassifiable while 19 (2,4%) were found to be untypable. (c.f. Table 3.31).

Universally, the most common pyocin inhibition pattern encountered is that of pyocin type 1 and such strains (161) together with those belonging to types 3, 5, 10 and U/T account for 58% (162) to 85%

(163) of all isolates. In this study 53,7% of the total isolates were type 1, type 5 and U/T made up less than 5% with types 3 and 10 not being isolated.

Although several sites of the patients were swabbed on several occasions, none of the patients had more than one pyocin type of *P. aeruginosa* in the same site. This is contrary to what was reported by Deighton *et al* (164) who found more than one pyocin type of *P. aeruginosa* from the same site in 15% of hospital patients and Heckman *et al* (163) in 13% of similar patients. Govan and Gillies (152) suggested that in the majority of cases, the presence of more than one type in a specimen is due to multiple infection and reflects the high incidence of different strains of *P. aeruginosa* in the hospital environment. This would not appear to be true in this study.

Pyocin typing is a valuable aid in epidemiological studies of *P. aeruginosa*. The technique has been used to determine the incidence of exogenously and endogenously acquired infections and to elucidate the sources, reservoirs and mode of spread of the organism in the hospital environment. Outbreaks involving many patients in a short period of time are easily recognized. The use of pyocin typing has implicated faulty sterilization techniques and many inanimate vehicles in the spread of infections due to *P. aeruginosa* (165).

Govan (165) in an early application of the technique of pyocin typing, describes the detection of two long-term episodes of cross-infection in two adjacent wards. During a 16-month period, strains of pyocin type 35 accounted for 26 (72%) of the 36 cases of infection

with *P. aeruginosa* in unit A. Concurrently in unit B, strains of pyocin type 29 were isolated from 23 (70%) of the 33 similar cases of infection. Improper sanitization of urine bottles and milk feed stoppers, was the cause of the outbreaks. Duncan and Booth (166) encountered an outbreak of infection due to type 1. The causal organisms were isolated from rubber urine collection bags and the surrounds of the physiotherapy treatment pool. All three outbreaks ceased following improved sanitization procedures.

With hospital equipment such as respirators, sterilization may be more apparent than real. Tinne *et al* (167) described an outbreak of respiratory tract infection in a cardiac surgery unit which affected seven cases with three deaths. All cases were due to pyocin type 10 strains and post-operative mechanical ventilation implicated. It was significant that the last three cases of infection occurred after "sterilization" of the respirator by disinfection and exposure to ethylene oxide. The unit was closed, sanitized and intricate equipment dismantled before exposure to ethylene oxide. On reopening the unit another case of respiratory tract infection with a type 10 strain was encountered raising doubts whether these measures had been effective. It was found, however, that this patient had been present in the unit during the outbreak. The organism was eradicated from this patient's sputum by chemotherapy but not before cross-infection had occurred to the patient in the adjoining bed.

Fierer *et al* (285) in the first reported use of pyocin typing in the U S A further emphasized the role of the infected patient and human carrier following successful sterilization of implicated apparatus. The original cause of an epidemic which involved 22

new-born infants and resulted in two deaths, was found to be delivery room resuscitation equipment that had been contaminated with *P. aeruginosa* via a wash-sink aerator. After disinfection, the causal strain was never again isolated in the nursery except from infected babies. Cross-infection through the contaminated hands of personnel was considered the most likely means of transmission to infants not resuscitated at birth and for the 12 new cases encountered after eradication of the causal organisms from the equipment.

In another report, Duncan and Booth (166) concluded from pyocin typing results obtained in a three year investigation, that endogenous rather than exogenous spread was the most common method of spread in *P. aeruginosa* infections in extended-care and urology wards. Patients acquire an increased faecal carriage of *P. aeruginosa* following admission to hospital (168) and pyocin typing has demonstrated that faecal carriage may lead to endogenous infection (164, 165).

In this study 38 strains of gentamicin resistant *P. aeruginosa* were isolated from the faeces of 130 patients. In addition 29 (10%) of the 290 staff members screened were found to be faecal carriers of GRGNB. The acquisition of gentamicin-resistant pseudomonads and enterobacteria by patients admitted to this hospital will be discussed in more detail at a later stage.

Valuable use of pyocin typing to determine the sites of colonization of patients with the same pyocin type was made by Heckman *et al* (163). In addition to a study of sites and patterns of colonization, results from 17 patients demonstrated correlation between clinical

diagnosis and the pyocin types of organisms isolated from one or more ante-mortem sites and post-mortem material.

Shulman *et al* (54) used pyocin typing to demonstrate the emergence and spread, in a burns unit, of a gentamicin-resistant strain of *P. aeruginosa* belonging to pyocin type 5. The gentamicin-resistant strain did not spread to areas of the hospital which did not use gentamicin and the outbreak was dramatically reduced following discontinuation of the routine use of the antibiotic.

The hospital environment usually harbours many different pyocin types of *P. aeruginosa*. In this study, pyocin typing was used as one of the methods to characterize this organism in order to investigate and control infections. Except in episodes of cross-infection, the distribution of pyocin types encountered in infections in a unit, over a period of time, follows no regular pattern as stated earlier in this study. The epidemiology of nosocomial infections due to *P. aeruginosa* is therefore different from similar staphylococcal infections where a large proportion of infections are caused by a few resident "hospital" staphylococci.

4.2 Phage Typing

Phage typing is a method of bacterial differentiation based upon the sensitivity of strains to certain bacteriophages. Phage typing fulfils a valuable function when investigating outbreaks or epidemics, since it allows one to exclude apparent sources and reveal the real source of infection. Many examples have been cited in this study showing that in epidemics or epidemic foci with a single source of

infection, the same phage type is isolated from all patients and contacts. Phage typing within the focus also makes it possible to establish the extent of the epidemic area, the persistence in the carriers of the pathogenic agent, and differentiation of the epidemic strains from those of carriers or sporadic cases unrelated to the local outbreak.

In this study, 789 gentamicin resistant strains of *Pseudomonas aeruginosa* were phage typed; using a set of 21 phages.

Table 3.31 shows that 12 phage types were identified from a total of 789 strains investigated. Of these 424 (53,7%) were phage type F7. The other phage types isolated were : type 24 (11,3%), type 352 (10,6%), type 16 (7,0%), type 31 (5,1%) and types 31/68/109/21/M6/1214 and 7 which together accounted for less than 5% of isolates.

P. aeruginosa phage type F7 was the commonest type and found in all the wards studied. This type was isolated from clinical specimens, inanimate environment and also from the staff members. This type referred to as *P. aeruginosa* K E H strain 1 seems to be the resident flora of this hospital, since it was isolated throughout the course of this study (c.f. Tables 3.1-3.30).

The selection of certain strains with an increased ability to spread and pathogenicity, often resistant to antibiotics in hospital conditions, is more easily followed up by phage typing. Meitert and Meitert (169) observed that *P. aeruginosa* strains belonging to the same phage type, isolated from hospital infections, were associated with increased properties of spread and virulence. Vieu,

cited by Meitert (169), showed that the diversity of *P. aeruginosa* phage types, which appear endemically in hospitals, contrast with the uniformity and virulence of the phage types isolated in extensive hospital outbreaks. Strains isolated from endemic areas offer a greater variety of phage types than epidemic strains (169). This fact in many instances discounts the possibility of direct epidemiological relationships. There are, however, certain situations in which sporadic apparently unrelated cases may be correlated by phage typing. Bercovici *et al*, cited by Meitert(169) reported the same *Shigella boydii* phage type in several cases of acute enteritis occuring in different districts of a town. These followed the consumption of a meat jelly prepared by a chronic carrier from whom the same type was isolated. In the present study, 12 phage types of *P. aeruginosa*, were isolated from patients, inanimate environment and staff members who acted as carriers and routes of transmission. Their relationship in the spread of nosocomial infections in this hospital will be discussed at a later stage.

The difficulty in comparing phage types is due to the different methodologies used at different centres, to the variability *in vitro* and in view of the phage types under the influence of various ecological factors, and to changes in the properties of certain typing phages. The diversity of the lytic spectra of pseudomonas phages, also make it difficult to compare the data repeated from different countries even with the same phage typing set.

4.3 Serotyping

Serotyping of *P. aeruginosa* is another method by which nosocomial spread of this organism can be followed. *P. aeruginosa* investigations carried out by serotyping based upon O-antigens have shown that useful information may be obtained by this method (Habs, 1957., Meitert *et al*,1974; Sandvik, 1960; Lanyi, 1970; Fisher *et al*, 1969; Homma, 1971; - all cited by Meitert and Meitert (169).

Table 4.1 cited by Lanyi and Bergan (170) shows the incidence of *P. aeruginosa* serogroups in miscellaneous non-faecal pathological specimens. It can be seen from this table that the most frequently encountered serogroup in extra-intestinal pathological specimens all over the world is O:2 (18,6-37,1%, average 29,9%). Low values were described by Wretlind *et al* (1973) (15,9%), and by Homma *et al* (1970) (11,6%), as well as very high incidence was shown by Meitert and Meitert (1966)(66,6%) in Rumania. Serogroup O:6, occurred in 7,8-32,2% (average 17,1%). Serogroup O:6 is more frequent than O:2 in Norway, whereas O:2 constitutes only a quarter of the strainsin Poland (Bergan, 1972). A relatively low incidence of O:6 has been seen in India (7,8%) and Japan (9,6%). Serogroup O:11, in turn has been found considerably more frequently in India (23-24,1%), in Japan (16,3%) and in the U S A (8,9-17,7%) than in Europe (3,7-7,1%). Among the rest, serogroups O:1, O:3 and O:7 occur with an average frequency of not more than five per cent.

In this study, 789 strains of *P. aeruginosa* were serotyped. Of these 67 strains were of faecal origin whilst 722 strains were isolated from miscellaneous non-faecal human specimens and the inanimate environment. The most frequently encountered serotype in this study

Table 4.1 Incidence (%) of *P. aeruginosa* Serogroups in Miscellaneous Non-faecal Human Specimens

Country and reference	No. of strains	Distribution according to serogroups (%)															
		1	2	3	4	6	7	9	10	11	12	13	14	15	SA	PA	ND
German Federal Republic (Kleinmaier) and Quincke, 1959)	301	10.6	18.6	5.6	13.0	16.6	8.0	0.7	2.0	3.7	2.3	.	.	.	6.3	0	12.6
USA (Verder & Evans 1961)	90	12.2	36.8	3.3	.	24.4	2.2	3.3	.	8.9	6.7	12.0	.	.	0	0	0
Rumania (Meitert and Meitert 1966)	364	.	66.6	12.9	2.5	12.7	2.7	.	.	.	0.5	.	0.5	.	0	0	1.6
Hungary (Lanyi, 1966/67)	827	3.5	37.1	9.4	1.5	14.1	12.9	2.3	0.6	7.1	0	.	.	0	0.7	10.2	0.6
USA (Fisher *et al;* 1969)‡	342	10.3	30.1	.	.	21.8	5.5	.	8.6	17.7	.	.	.	.	.	.	6.0
Denmark (Mikkelsen, 1970)	767	5.0	28.3	6.6	0.5	15.4	3.1	2.9	4.7	6.4	0	0.1	.	.	12.1	0	14.9
Japan (Hamma *et al;* 1970)§	915	10.9	11.6	6.6	2.5	9.6	1.6	4.5	8.1	16.3	.	.	.	.	1.0	15.5	11.8
USA (Adler & Finland, 1971)	83	12.0	36.1	.	.	14.5	4.8	.	2.4	13.3	.	.	.	.	.	.	16.9
India (Agarwal *et al;* 1972)‡	526	4.9	26.2	5.7	.	7.8	7.9	1.0	0.8	23.0	6.0	0.2	.	.	0	0	16.5
USA (Moody *et al;* 1972)	742	4.6	28.1	.	.	25.2	19.9	.	6.6	15.1	.	.	.	.	0	0	0.5
Poland (Bergan, 1972a)	302	1.7	23.5	3.3	1.0	11.9	1.0	2.6	1.7	2.0	0	0.7	1.7	18.9	13.6	0	15.2
Sweden (Wretlind *et al;* 1973)	189	7.9	15.9	7.9	3.7	32.2	4.2	1.1	4.8	6.9	1.1	7.4	.	.	0	3.2	3.7
South Africa (this study)	789	0	0	0	0	11.3	0	2.3	0	56.0	0	0	.	.	3.9	17.6	8.9

‡ Source of strains not stated.
§ Including faecal strains.
Abbreviations: SA = self-agglutinable, PA = polyagglutinable, ND = not determined or non-groupable according to the compiled scheme, . = not tested

was 0:11 (56%) (c.f. Table 3.31). This serotype is almost twice as common as that seen in India by Agarwal *et al*, (1972) and almost four times that seen in U S A. (Fisher *et al*, 1969). The next most common type seen in this study was 0:6 (11,3%). This is also the second most frequently encountered serotype to 0:2 in extra-intestinal pathological specimens all over the world (170). The highest incidence of 0:6 has been reported in Sweden by Wretlind *et al* (1973) whilst relatively low incidence of 0:6 has been seen in India (7,8%) and Japan (9,6.). The most frequently encountered serogroup in non-faecal human specimens all over the world is 0:2, which was not isolated in this study. One hundred and thirty-nine (17,6%) of the 789 strains were found to be poly-agglutinable whilst 101 (12,8%) were non-typable. It must be noted that the comparison in the incidence of serogroups made here included *P. aeruginosa* isolates from faecal, miscellaneous non-faecal human specimens and inanimate environment.

No apparent relationship was found between serogroups and either source of specimen or characteristics such as mucoid appearance and pigment production, in this study. A similar finding was also reported by Sato and Diena (171). Denis and Godeau (1972) and Yoshioka *et al* (1970) found some serotypes to be somewhat more resistant to antibiotics. Others have revealed that there is no association between serogroups and susceptibility to antibiotics, pathogenicity, site of infection, or biochemical or cultural characteristics (Adler and Finland, 1971; Bergan, 1972; Klyhn and Gorrill, 1967; Kohler, 1957; Lanyi, 1969; Michel-Briand *et al*; 1975 - all cited by Lanyi and Bergan (170).

In this study all *P. aeruginosa* strains resistant to gentamicin and other antibiotics were serotyped. The antibiotic susceptibility patterns appear to show no association between serogroups and a particular characteristic of the organisms, thus showing that this study is in agreement with the findings of others.

Correlation between typing methods

There appears to be little correlation between results of phage or pyocin typing, regardless of the typing scheme employed, but considerable correlation does exist between certain pyocin types and serotypes (Sjoberg and Lindberg, 1968; Govan, 1968; Farmer and Herman, 1969; Bergan, 1973 - all cited by Govan (161). Govan (165) and Siem (162) found a significant correlation between strains belonging to pyocin type 3 and serotype 6. Siem (162) noted that of 149 strains of the unusual pyocin type 2, 131 belonged to serotype 6 and Govan (165) found that all nine pyocin type 17 strains examined and all three pyocin type 19 strains also belonged to serotype 6. Siem also confirmed that 10 of 11 strains of pyocin type 11 belonged to serotype 1. Initially there appeared to be little correlation between strains of pyocin type 1 and any particular serotype. However, when such strains were allocated to subtypes on the basis of their activity against indicator strains A-E, certain correlations emerged. Csiszar and Lanyi (1970) - cited by Govan (161), observed that pyocin types 1/c and 1/d accounted for 80% of all strains of serotype 2; 15 of 17 strains belonging to pyocin type 1/f were of serotype 9 and 15 of 34 strains belonging to pyocin type 1/b were of serotype 11. Al-Dujaili and Harris (172) noted that 11 of 15 strains belonging to pyocin type 1/h were

of serotype 11.

In the present study, the subtypes of pyocin type 1 were not done but it was found that 424 (53,7%) of the 789 strains typed were pyocin type 1 and serotype 11. The unusual pyocin type 2 was not identified in this study but the correlation between pyocin type 17 and pyocin type 19 strains belonging to serotype 6 was evident. Eighty-nine of pyocin type 19 belonged to serotype 6 and 84 of pyocin type 17 strains belonged to serotype 6 and 10. These results correlated well with the findings of Al-Dujaili and Harris (172) and Govan (165).

Typing methods have been instrumental in the elucidation of nosocomial patterns of infection in many hospitals.

Knights *et al* (173) employed all three typing methods to characterize the epidemiology within a neonatal unit. The typing methods enabled an analysis of the identification of the reservoirs of infection and pathways of nosocomial spread. Cross-infection of four strains implicated several wards and inanimate sources such as nursery washbasins, sinks , sluices and cleaning equipment. Measures instituted to eradicate pseudomonas, were so successful that no milieu or patient yielded pseudomonas for a subsequent 8-month period.

In another study, Ayliffe *et al* (174) in a series of cases of pseudomonas meningitis after neurosurgery, recovered the same phage pattern, pyocin type and serogroup on a shaving brush used for depilation prior to surgery. The typing methods were instrumental in establishing the causative relationship and in eliminating a

number of sources such as staff, hand creams, sinks, soaps, trays, aseptic bottles, urine bottles, and another depilation brush. Similarly, intraocular pseudomonas infections arising after eye operations were caused by a contaminated saline solution used to moisten the cornea during operations (175). The saline had been contaminated from a sink during washing.

The typing methods employed in this study, enabled an analysis of the identification of the reservoirs of infection and pathways of nosocomial spread of gentamicin-resistant *P. aeruginosa*, an organism most frequently isolated during the course of this study. The role of this organism and other gentamicin resistant gram negative bacilli in the spread of hospital acquired infections is considered in more detail in the discussion that follows.

4.4 Antibiotic susceptibility patterns of GRGNB

A total of 1703 gentamicin resistant gram negative bacilli isolated from patients, staff and inanimate environment were tested against eight antibiotics: gentamicin, kanamycin, tobramycin, streptomycin, carbenicillin, polymyxin B, amikacin and sisomicin. (c.f. Table 3.31).

Of the 789 *P. aeruginosa* isolates tested, all were resistant to gentamicin, tobramycin, kanamycin, streptomycin and sisomicin. Twenty-nine per cent were resistant to carbenicillin and 13% to amikacin. Eight per cent were intermediately sensitive to carbenicillin and 5% to amikacin. All the isolates tested against polymyxin B were sensitive to that drug.

Forty-two *P. cepacia* isolates were tested against the same range of antibiotics. All were resistant to the eight antibiotics.

A total of 13 *P. stutzeri* isolates were found to be resistant to gentamicin, tobramycin, kanamycin, streptomycin, carbenicillin and sisomicin. All the isolates were sensitive to polymyxin B and amikacin.

Of the 232 *K. pneumoniae* isolates tested, all were resistant to gentamicin, tobramycin, kanamycin, streptomycin and sisomicin. All the isolates were sensitive to polymyxin B and amikacin. A similar susceptibility pattern was shown by *K. aerogenes* except that all 35 isolates were intermediately sensitive to amikacin.

A total of 189 isolates of *A. anitratus*, 88 *E.coli*, 21 *E. aerogenes* and 16 isoaltes of *C. freundii*, showed similar antibiotic susceptibility pattern to *K. pneumoniae*.

All seventy-two isolates of *P. rettgeri*, 12 *P. mirabilis* and 49 isolates of *P. stuartii* were resistant to gentamicin, tobramycin, kanamycin, streptomycin, carbenicillin, polymyxin B and sisomicin but sensitive to amikacin. A similar antibiotic susceptibility pattern was shown by 36 isolates of *P. morganii* except that the organisms were sensitive to both carbenicillin and amikacin. Seventeen isolates of *E. cloacae* and 16 isolates of *A. faecalis* were resistant to all the antibiotics except polymyxin B.

All 76 isolates of *Flavobacterium meningosepticum* were resistant to all the antibiotics tested.

Thus, these GRGNB displayed multiple antibiotic resistant patterns.

Tobramycin, kanamycin, gentamicin, streptomycin and sisomicin were always inactive, whereas most isolates were susceptible *in vitro* to amikacin and polymyxin B. Varying degrees of susceptibility was displayed against carbenicillin.

In order to indicate the relative activities of the various antimicrobial agents, cumulative percentages are given in Tables 3.32.1 - 3.32.5. This gives some indication of the *in vitro* "susceptibility" or "resistance" of the organisms.

Tobramycin has been reported to be two to four times more active than gentamicin against *P. aeruginosa* (176). In this study it was found that gentamicin resistant *P. aeruginosa* were also resistant to tobramycin. There are differing reports from other laboratories on the activity of sisomicin. Sisomicin is said to be more active than gentamicin or tobramycin against *E. coli* (177), but inferior activity has been reported by another group (178). In this study, all 88 isolates of *E. coli* were resistant to gentamicin, tobramycin and sisomicin. Sisomicin MIC's were generally higher than those of gentamicin and since attainable serum levels of these two antibiotics are similar, there seems little point in choosing sisomicin.

Amikacin has been reported to be active against gentamicin-resistant *P. aeruginosa*, *Providencia species* and *Serratia marcescens* (73,179) and showed considerable activity againt gentamicin-resistant gram negative bacilli in this study. Clinical trials with amikacin have given promising results (180-182) , and this antibiotic has an important role in chemotherapy, if infections with gentamicin-resistant organisms, especially if they become more common. In this study, 102 (12,9%) isolates of *P. aeruginosa* were resistant to

amikacin and of the 1703 gentamicin resistant gram negative bacilli tested, 253 (14,9%) were resistant to amikacin. The finding of high level cross resistance to amikacin among gram-negative bacteria, though reported has not been common worldwide. Block (89) in South Africa, examined gentamicin-resistant strains and found cross-resistance to amikacin in only 6,9%; Yu (183) at the Mayo Clinic found 7%; Reynolds (184) in England 19%; and Seligman (185) in New York 6%. In Switzerland (186) among aminoglycoside-resistant gram-negative bacteria, 70% were gentamicin-resistant and 8% were amikacin resistant. A high degree of amikacin cross-resistance among *P. aeruginosa* has also been reported. John *et al* (187) in a study of nosocomial infections at the Medical University Hospital of South Carolina found 61% of gentamicin resistant *P. aeruginosa* were also resistant to amikacin. A similar finding of amikacin resistance among *P. aeruginosa* has also been reported from Massachusetts General Hospital (188) whereas the Enterobacteriaceae at that hospital showed minimal cross-resistance.

In this study 844 isolates of pseudomonads and 859 isolates of enterobacteria were found to be multi-resistant.

Other workers have also reported the isolation of multiple antibiotic resistant organisms. Martin *et al* (59) described an outbreak in which they showed that all 19 GRGNB isolates from 14 patients were resistant to streptomycin, kanamycin, neomycin, ampicillin, tetracycline, and chloramphenicol. Fifteen were sensitive to both cephalothin and the polymyxins, two were cephalothin-sensitive and polymyxin-resistant, and two were cephalothin-resistant and polymyxin-sensitive.

The susceptibility pattern of 50 GRGNB to 10 antimicrobial agents were assessed by Block (89). Forty two percent were resistant to ampicillin, carbenicillin, cephalothin, tetracycline, chloramphenicol, sulphonamides, co-trimoxazole, streptomycin and kanamycin. With the exception of 5 isolates of *Proteus species*, all were susceptible to the polymyxin group of antibiotics. Ten percent were susceptible to carbenicillin, 14% to cephalothin, 22% to tetracycline, 10% to chloramphenicol and 26% to co-trimoxazole in various combinations. In the same study 259 clinical isolates of gentamicin-resistant gram negative bacilli revealed 99,2% cross resistance with tobramycin and 6,9% with amikacin.

Casewell and Talsania (90) tested 108 strains of gentamicin resistant *K. aerogenes* selected from 12 hospitals in six countries. They found that resistance to many antibiotics in addition to gentamicin, was common. Ninety-nine strains were resistant to at least 10, and 31 to at least 15 of the 24 antimicrobial agents tested. Of the 108 strains, 100 were resistant to streptomycin, 96 to kanamycin, 85 to tobramycin, 81 to neomycin and 4 to amikacin. All strains were resistant to carbenicillin and ampicillin.

French *et al* (64) reported a hospital outbreak of antibiotic resistant *Acinetobacter anitratus*. The organism was resistant to many other antibiotics including streptomycin, kanamycin and gentamicin with MIC's of more than 128 µg/ml.

Of the 179 strains of *E. coli* tested by Noy *et al* (85), 66% were resistant to streptomycin, 60% to carbenicillin and 15% to kanamycin. The 236 *Klebsiella species* examined were mostly resistant to

ampicillin (97%) and carbenicillin (80%).

Tube dilution susceptibility tests of GRGNB carried out against aminoglycosides by Noriega *et al* (130), showed that 90% of *Klebsiella* and *Enterobacter species* were highly resistant to gentamicin, kanamycin and tobramycin. Seventy percent of *Klebsiella* strains were sensitive to streptomycin, whereas the majority of enterobacter strains were resistant.

John *et al* (187) in a study on the characteristics of gentamicin resistance in nosocomial infections, found *P. aeruginosa* to demonstrate a high cross resistance to amikacin (61%) and also to tobramycin (58%). The resistance pattern of several isolates was unusual, characterised by high level gentamicin and amikacin resistance (>100 µg/ml). Cross resistance to amikacin and tobramycin was also seen in six of 12 gentamicin-resistant enterobacteria.

Thus, the results shown by this study and other workers indicate that a clear association exists between gentamicin resistance and multiple antibiotic resistance.

4.5 Antibiotic Usage

During the last 20 years, there has been an increasing clinical awareness of hospital infections caused by bacteria resistant to several antibiotics available for normal use, and even to some antibiotics in various stages of development. Antibiotic resistant bacterial populations arise in man and animals by the use of antibiotics whether for therapy, growth promotion or prophylaxis (45).

Smith (42), Guinee (37), and Lebek (39) have shown that widespread usage of antibiotics in animal feeds has played a significant role in encouraging the emergence of resistant bacteria while Walton (47) and Aronson (36) suggested that the indiscriminate use of antibiotics in human medicine provides a major selective force for the emergence of resistant strains.

There has been some controversy over the relationship of the use of antimicrobial agents and the prevalence of antimicrobial resistance in aerobic gram-negative bacteria. There is evidence that the factors responsible for the antimicrobial resistance of *Enterobacteriaceae* were present before the introduction of some antimicrobial agents (189). This is supported by the presence of an R-plasmid in an *E. coli* culture lyophilized before drugs were widely used (190) and by the present rarity of these plasmids in the bacterial flora of humans and animals living in drug-free environments (40,41). Resistant bacteria were discovered in Japan in 1959 (21,26). Shortly afterwards they were isolated from patients in Britain (191) where their incidence among *S. typhimurium* isolates increased from 21 per cent in 1964 to 61 percent in 1965(16).

Today resistant bacteria are abundant among patients and in the general population in many parts of the world (16,19,22,23,33,37, 38,39,192).

Although a few studies have indicated that the prevalence of antimicrobial resistance in commonly encountered gram-negative bacteria has not increased(193,194), most reports have indicated the opposite (16,195-197).

The emergence of resistant organisms has been clearly related, for several bacterial species, to the intensive use of antimicrobial agents. Finland, Jones and Barnes (198) reported that the use of antimicrobial agents in hospitals has produced changes in the host and his bacterial flora "which seem to enhance the number, pathogenicity, and invasiveness of various micro-organisms which, under ordinary circumstances, appear to be rather benign".

To appreciate the potential impact of antibiotics on bacteria, one should be aware of the quantities of antimicrobial drugs used annually. In the U S A the annual usage of antibiotics rose from 2,2 million kilograms in 1960 to 8,2 million kilograms in 1970, an increase of almost 400 per cent (48). In 1967 over 360 thousand kilograms of antibiotics were used in human and veterinary medicine in Britain (49).

Table 3.36 shows the amount of parenteral gentamicin used at King Edward VIII Hospital from 1979 to 1983. The use of parenteral gentamicin has almost trebled over the last five years. Gentamicin in the form of drops, ointment and powder are not included in this table. The same table also shows the use of amikacin in this hospital, which has more than doubled over the last 3 years.

Gentamicin is freely available to the doctors whereas amikacin has to be motivated for in this hospital. The human bacterial flora is subjected to this tremendous antibiotic selection pressure primarily under conditions of therapy.

Isenberg and Berkman (5) have claimed that in an average 300 bed hospital, the process of clearing the needle, by expelling a small portion of the syringe contents prior to injection, adds 15 to 30 litres of high potency antimicrobial agents to the environment annually. King Edward VIII Hospital is a 2000 bed hospital and if one considers the above data, there will be more than six times the amount of high potency antimicrobial agents added to the environment of this hospital, annually. It must be expected therefore that organisms isolated from the hospital environment would be drug resistant. It is not surprising therefore that several studies have reported a greater incidence of antibiotic resistant bacteria in hospitals as compared to the healthy population (35,38,39).

In this study, 40% of the patients had received treatment with gentamicin and 60% had received other antibiotics, including other aminoglycosides and carbenicillin. The patients who harboured gentamicin-resistant gram-negative bacilli in this study, had underlying illnesses similar to those reported by others (63,83,103, 123,125,223), most commonly extensive burns, respiratory failure, indwelling bladder catheters, serious accident or surgical wounds and renal failure. Also common to these patients were exposure to parenteral gentamicin, exposure to other broad-spectrum antibiotics and hospitalization for over 10 days. The duration of exposure to aminoglycoside therapy was not in itself related to the acquisition

of resistant organisms. Some patients did have repeated exposure to aminoglycosides, and such patients may be appropriate subjects to study prospectively in order to elucidate the relation between duration of antibiotic exposure and emergence of resistance.

The relationship between prior antibiotic therapy (including aminoglycosides), and the acquisition of gentamicin resistant gram-negative bacilli by patients after admission to King Edward VIII Hospital was studied by Pillay (199). In this study 50 patients out of a total of 116 acquired GRGNB after admission to this hospital. Forty seven of the 116 patients did not acquire GRGNB throughout their stay in hospital. Forty four of the acquirers had received prior antibotic therapy as compared to 28 of the non-acquirers who had received antibiotics. Twenty seven of the acquirers and 11 of the non-acquirers had been treated with aminoglycosides. This study demonstrated a significant association between prior aminoglycoside therapy and the acquisition of gentamicin resistant gram negative bacilli at this hospital.

Starkey and Gregory (196) also found a direct relationship between the use of antimicrobial agents and the prevalence of antimicrobial resistance in a veteran's hospital in Montreal between 1961 and 1970. Finalnd (195) was able to relate antimicrobial usage to the emergence of resistant strains of *Klebsiella*. In addition, there have been a number of reports of the development of kanamycin-resistant gram-negative bacillary colonisation and infection, primarily in new born nurseries, related to heavy use of broad spectrum antimicrobial agents (200-204).

Antibiotic use has also been implicated as one of the causes of an

outbreak of gentamicin-resistant *Klebsiella* infections that occurred in a general hospital in adults (50), and for an epidemic of multi-drug resistant *Klebsiella* infection in a regional neurosurgical intensive care unit in Scotland (205). In the latter outbreak, eight patients died of *Klebsiella* meningitis and three patients died of *Klebsiella* pneumonia before the epidemic was stopped by complete withdrawl of all antimicrobial use in the unit.

In a study of a nosocomial outbreak of multi-drug resistant *Klebsiella*, Gardner and Smith (206) showed that environmental selection by antimicrobial use and colonization of patients with resistant organisms are of far greater epidemiologic significance than transfer of R-plasmids in the gastrointestinal tract. Use of sulphonamides and aminoglycosides, especially topical preparations which are often used in treating burns patients, and carbenicillin has been associated with increasing emergence of antimicrobial-resistant strains of *Enterobacteriaceae* and *Pseudomonas aeruginosa* (86,207,208).

A number of studies have also demonstrated that colonization of skin surfaces, oropharynx and the gastrointestinal tract with gram-negative bacilli in hospitalised patients is strongly associated with antimicrobial use (121,209).

Rose and Schrier (210) compared 50 hospitalized patients treated with antibiotics with 50 untreated patients and noted a significant increase in the *Klebsiella* intestinal carrier rate during the hospital stay only among those patients receiving antibiotics. Farmer (211), in a prospective study of colonization of the upper

respiratory tract of premature infants, noted a 63% *Klebsiella* colonization rate for the 54 infants receiving antibiotics and only a 16% colonization rate among 51 patients not receiving antibiotics. Winterbauer, Truck and Petersdorf (212) noted that only 13% of patients not receiving antibiotics became intestinal carriers of certain serotypes of *E. coli* (04,06,075), whereas 37% of patients on broad-spectrum or multiple antibiotics acquired such strains.

An increased frequency of antibiotic resistant *E. coli* and *Klebsiella species* in the faeces of babies treated with antibiotics than of untreated babies was reported by Noy *et al* (85). In the present study 17 isolates of GRGNB were found in the faeces of 10 babies in the nursery ward. (c.f. Table 3.33). Of the 17 isolates, 3 were *P. aeruginosa* K E H strain 1, 10 *K. pneumoniae* and 4 *E. coli*. Three of these babies were on gentamicin and the rest were on other broad spectrum antibiotics.

Noriega *et al* (130) reported that of 20 patients treated with antibiotics other than gentamicin, seven had GRGNB in their stool. Of 22 patients treated with gentamicin for at least five days, 15 were faecal carriers of GRGNB.

Thirty-four episodes of bacteremias caused by GRGNB at the University of Virginia Hospital was reported by Guerrant *et al* (123). All except one of these bacteremic patients had received prior antibiotics. Nineteen of these patients had actually received gentamicin and 14 had received other antibiotics.

In addition to prior exposure to antibiotic therapy and various procedures, the severity of the patient's illness also appears to

influence the likelihood of bacterial colonization. Johanson, Pierce, and Sanford (279) demonstrated a significantly greater pharyngeal carrier rate of gram-negative bacilli among moribund patients than among less ill patients, correcting for previous exposure to antibiotic and respiratory therapy. The data in the present study do not permit adequate evaluation of this possibility.

There is little doubt that antimicrobial agents are both misused and overused. The widespread use of antibiotics for prophylaxis against infections has been a major contributing factor to the emergence of resistant bacteria. The use of polymyxin B aerosol to prevent colonisation and infection with *P. aeruginosa* in an intensive care unit was reported by Feeley *et al* (213). Although the incidence of colonisation and infection due to *P. aeruginosa* was significantly reduced, colonization and serious infection due to polymyxin-resistant organisms emerged. *Flavobacteria*, *Serratia* and *Streptococcus faecalis* were responsible for most of these infections and these organisms were also associated with a high mortality rate.

Broad spectrum oral antibiotics are often used in general practice with inadequate indication e.g. the use of ampicillin, tetracycline or cotrimoxazole to treat influenza or the common cold. In addition to the waste and expense, this practice is often associated with adverse side effects. While the indiscriminant use of antimicrobial agents in general practice has had only limited effects on the general bacterial ecology, the reverse has been true in hospitals, resulting in the gram-negative enteric bacteria largely replacing the staphylococcus as the leading cause of bacteremia and fatality (215).

Studies in North America have found that 13-35% of patients receive

antimicrobial agents during hospitalization, accounting for about one-third of hospital drug costs (216-218, 220). As many as one-half of the patients receiving antimicrobial therapy had not had cultures taken and, in one study by Scheckler and Bennett (219), up to 70% of patients receiving antimicrobial therapy had no evidence of infection. Antimicrobial use was found to be irrational or inappropriate in 38 to 66% of instances (216,218,220). Up to 80% of irrational use was given as prophylaxis with the vast majority of this type of use occurring on surgical wards. Roberts and Visconti (216) found that 77% of their hospital's antimicrobial cost was spent for irrational therapy. In addition, this therapy was responsible for 92% of all patients who experienced adverse drug effects.

Aronson (36) cites a survey in which the justification for clinical use of antibacterial drugs was evaluated. Of 340 patients receiving antibacterial drugs, 13% of the therapies were judged rational, 22% questionable and 66% irrational. He also questions the concept of giving antibiotics to be on the "safe side" and states that "the routine prophylactic use of antibacterial drugs in simple surgical procedures is now generally accepted as misuse of this group of drugs".

Neu and Howrey (221) demonstrated deficiencies of knowledge in the use of antimicrobial agents by North American physicians, especially for physicians in practice for 15 or more years. This phenomenon is probably world-wide and is likely related to the diversity and ready availability of new antimicrobial agents and the aggressive and often effective "educational" programmes offered by the pharmaceutical industry.

The widespread and often inappropriate use of antimicrobial agents

has resulted in the emergence of antibiotic resistant bacteria and has contributed to the increased incidence of nosocomial infections, especially those due to gram-negative bacilli.

4.6 Sources of GRGNB

These will be disucssed under the following main headings :

A. Patients

B. Staff and Boarder mothers

C. Environment

A. Patients

It has been estimated that 11-14% of patients have pseudomonas in the stool at admission and an additional 10-17% acquire it during the course of their hospitalization (168). One possible source by which *P.aeruginosa* may be acquired is food. Clinical isolates have been traced by pyocin typing to vegetables in hospital kitchens (222). Simon-Pujol *et al* (67) also isolated GRGNB from foods. In the present study, three strains of *P. aeruginosa* and three strains of gentamicin-resistant enterobacteria were isolated from the food of six patients (c.f. Table 3.34). The *P. aeruginosa* and the enterobacteria isolates from food showed similar phage patterns, identity in serogroup, pyocin type and antibiotic susceptibility patterns to that isolated from the faeces of these patients.

The highest number of GRGNB were obtained from the faeces (141) and this was followed by urine (87) (c.f. Table 3.33). Gentamicin resistant enterobacteria and pseudomonads isolated from the faeces and urine of many patients showed similar antibiotic susceptibility patterns, serotypes, pyocin types and phage types (c.f. Table 3.31).

This suggests that these patients might have developed urinary tract infections from their own intestinal flora i.e. autoinfection.

The influence of the hospital milieu on disease is also reflected in the finding that out-patients have a three-fold lesser probability of faecal pseudomonas carriage than warded patients (168). In this study 38 strains of gentamicin resistant *P. aeruginosa* were isolated from faeces and 42 strains from urine. The majority of these were identical in phage, sero and pyocin types suggesting spread between patients.

Shooter *et al* (168) used phage, pyocin and serological typing to show that pseudomonas may spread from the bowel of one patient to another. Strains were only considered related when there were similar phage patterns, identity in serogroup and pyocine type.

Jellard and Churcher (284), in investigating an outbreak which had lasted 16 months and in which seven patients died, found that the main reservoir of infection was babies' faeces and that these were formidable difficulties in preventing spread from baby to baby when the same nurses were responsible for both changing and feeding the babies.

The sources of infections due to gentamicin-resistant enterobacteria and pseudomonads in this study were similar to those reported by other workers (83,77,121,223,284). The majority of patients showed infection and carriage at the same time and it was not possible to decide whether carriage preceeded infection or vice versa. The significance of colonization of the respiratory tract of patients is discussed later.

B. Staff and Boarder Mothers

The role of hospital personnel in patient to patient spread of antibiotic resistant organisms has been well documented (74,224, 225).

In this study also, the bacterial species isolated from the staff members were similar to patient strains.

Culturing hands has been extensively used to search for reservoirs of epidemic-causing organisms and as a surveillance technique to evaluate general levels of cleanliness in the environment. Carriage of gram-negative bacilli on hands of hospital personnel has often been implicated as a mode of spread within hospitals (225-228).

Cultures of finger tip impressions of 310 doctors, nursing staff and cleaners revealed that 78 (25,2%) were positive for GRGNB. Six of the staff members had GRGNB on thier hands before, and the other 72 had positive cultures 2 hours after commencing duty (c.f. Table 3.35). The bacterial species isolated were *P. aeruginosa*, *K. pneumoniae*, *P. stuartii*, *A. anitratus*, *Flavo. meningosepticum*, *E. coli*, *A. faecalis*, *C. freundii* and *E. cloacae*. All of the isolates showed similar antibiotic susceptibility patterns to patient strains (c.f. Table 3.31).

An important vehicle for the transmission of nosocomial infections are the hands of members of the hospital staff. Hands cannot be sterilized, and the disinfectants which are most effective on inanimate objects are usually too toxic for application to the skin.

Moreover, the skin has its own microbial flora which is more difficult to remove or destroy than the micro-organisms present on inanimate objects. Pathogenic organisms deposited on the skin may become part of the normal cutaneous flora, or may only survive for a short time.

The importance of gram-negative bacilli on the skin seems to have been underestimated for a long time. It is well known that most gram-negative bacilli die through desiccation of the fluid in which they are deposited on the skin; they would, therefore, appear to be transient organisms, acquired through contamination by recent contact with a patient. More recent investigations, (227,228) however, have shown that gram-negative bacteria are often present on the skin in greater numbers than was previously thought, especially in humid sites, such as the axillae and the perineal region.

The evidence that implicates the role of hands in the transmission of antibiotic-resistant coliforms during hospital outbreaks of cross-colonization and cross-infection is increasing (74,79,224-226).

The reason why particular species, such as *Klebsiella* or *Acinetobacter* or certain strains within a species, are more likely to give rise to such outbreaks is poorly understood. If certain gram-negative bacilli survive better than others on the contaminated hand then this might explain why these organisms are more easily transferred from one patient to the next and initiate or sustain outbreaks. There is already some evidence to suggest that *Klebsiella species* survive better than other coliforms or *Pseudomonas aeruginosa* after inocu-

lation of the forearm (122,229) or of the finger tips (224,230) of normal subjects. Gentamicin sensitive strains of *Klebsiella aerogenes*, known to have caused hyperendemic colonization and infection of intensive care patients (233), survived for as long as 150 minutes after the inoculation of hands (74). Cooke, Edmondson and Starkey (231) have recently suggested that outbreak strains of *K. aerogenes* survive better than sporadic or environmental strains during the first 10 minutes after the inoculation of finger tips.

Hand carriage in about 20-30 per cent of the hospital personnel has been reported (225,227, 232), although isolation rates of 80% and more are mentioned in intensive care nurseries or burn unit personnel (226). In some outbreaks of hospital-associated infection by gram-negative bacilli, these organisms can be isolated from the hands of many of the nurses. Hands can easily be contaminated by contact with a patient, particularly if no special precautions are taken. Patient care and handling involve an important risk of acquiring micro-organisms. Changing a baby's diaper containing faeces or patting the buttocks of a baby with a clean diaper led to the acquisition of coliforms on the hands of the nurse in 44 of 49 experiments (234).

Mortimer *et al* (235) followed the acquisition of staphylococci by infants and nurses from an index infant, known to be a nasal carrier of a bacteriophage-typable strain, when the baby was touched or not touched by these nurses. They were able to demonstrate that the transmission of staphylococcal infections occurs mainly by physical

transfer on hands : the organisms were carried in the nose, transferred to the hands and from them to the patient (primary transfer), or the organisms were transmitted from an infected patient via the hands to another susceptible patient (secondary transfer).

Cultures of throat swabs and nasal swabs of 300 doctors, nurses and cleaners in this study, showed that 14 (4,7%) of the staff carried GRGNB in their throat and 8 (2,7%) in their nose (c.f. Table 3.35).

Maples and Towers (236) demonstrated that the hands could become contaminated from contact with a contaminated object, and that the contaminated hands could then contaminate other objects or tissues.

It is not easy to demonstrate that an infection originates from or is transmitted by hands. The observation that a pathogenic organism can be isolated from a nurse's hands does not prove that this strain has caused the infection. Even when an outbreak comes to an end following the introduction of rigorous hand washing before and after patient handling, the final result is determined by the sum of many factors, such as improved isolation techniques, treatment of linen and disinfection of instruments, which may have been introduced at the same time.

Many epidemics are described in which hands have probably served as a vehicle in the spread of infection (64,74,103,237,238).

Transmission of multidrug resistant *Acinetobacter anitratus* from patients' skin to staff hands was demonstrated by French *et al* (64).

Of the 38 staff working in affected wards, 11 had positive hand cultures. Two microbiologists investigating the outbreak also became hand carriers. Following prompt identification of new cases and closer attention to staff hand washing, no further cases were seen.

Noriega *et al*(130) took cultures of hands of 38 medical personnel without prior notice, and isolated gram-negative bacilli in 12 instances; four of these organisms were resistant to gentamicin.

Casewell and Phillips (74) found that 17% of the staff of an intensive care ward had *Klebsiella species* contaminating their hands. These strains could be related to serotypes infecting or colonizing patients in the ward on the same day. They identified some simple ward procedures that resulted in contamination of nurses' hands with 100-1000 *Klebsiella* per hand. These procedures were : lifting the patient, general nursing, physiotherapy, taking pressure, pulse and temperature, washing the patient, and touching the hand, groin, shoulder and tracheostomy. Handwashing with chlorhexidine hand cleanser reliably gave 98-100% reduction in hand counts, and the introduction of routine handwashing by staff before moving from one patient to the next was associated with a significant and sustained reduction in the number of patients colonized or infected with *Klebsiella species*.

In the present study, besides staff and patients, thirteen isolates of GRGNB were obtained from the hands of 10 boarder mothers in the nursery. The bacterial species isolated were *P. aeruginosa*, *K. pneumoniae* and *E. coli* (c.f. Tables 3.28 and 3.33). One of the

mothers had both *P. aeruginosa* and *K. pneumoniae* on her hands.

The isolates obtained from the mothers' hands were similar to those isolated from their babies. The three isolates of *P. aeruginosa* causing conjunctivitis in three babies were of the same sero, pyocin and phage types to that isolated from their mothers' hands. It is recommended that the mothers wash their hands before and after touching their babies.

Several examples in which hands were obviously implicated as a vehicle of cross-infection were cited in this study. Even if hand carriage or contamination is not the sole factor in the spread of nosocomial infections, it can be regarded as one of the most important factors. Handwashing and hand disinfection must, therefore, be regarded as primary measures, in the prevention of the transfer of infections (239).

Cultures of rectal swabs of 290 doctors, nurses and cleaners from the wards at K E H showed that 29 (10%) of the staff carried GRGNB intestinally (c.f. Table 3.35). None of the staff members were on any aminoglycoside antibiotic treatment except 12 staff members who were on either tetracycline, erythromycin or ampicillin for minor complaints. Two of the staff members who were intestinal carriers of gentamicin resistant *Klebsiella pneumoniae*, were also carriers of the same organism in their throat and nose.

A low intestinal carriage rate of 10% and a much higher hand carriage of 25,2% by hospital personnel has also been reported by numerous workers (121, 225). The failure to find a high prevalence of multidrug resistant intestinal carriers among the hospital personnel probably results at least partially, from their not receiving antibiotic therapy as suggested by Salzman, Clark and

Klemm (225).

The wearing of a protective gown or apron is an accepted part of isolation nursing technique. Disposable plastic aprons are cheap and impermeable to bacteria and water (240), but although protecting the probable area of maximum contamination, do not cover the arms, shoulders or back.

In this study, contact plates were used for sampling three areas, the front of the garment at bed height, the cuff of the sleeves and the shoulders. Although this method is less efficient than some others (242, 244) as the surface only is sampled, it is the only appropriate method for collecting large numbers of samples in an in-use situation.

Gentamicin-resistant gram negative bacilli were frequently isolated from staff clothing. A total of 80 isolates (25,8%) of GRGNB were obtained from the clothing of 310 staff members (c.f. Table 3.34). Bacteria were less frequently isolated on plastic aprons than on gowns or dresses, possibly because drying is more rapid on a plastic surface and organisms are more difficult to remove, as suggested by Babb, Davies and Ayliffe (243).

The role of protective clothing in preventing the spread of infection is clear from this and other studies (241-244).

The antibiotic susceptibility pattern of GRGNB isolates and the distribution of type strains from the staff members were similar to those isolated from the patients and their immediate environment in a particular ward.

C. Environment

Spread from the bowel to the hospital environment is inevitable both among adults and newborns (168). Shooter *et al* (168) recovered patient strains from water jugs, draw sheets, table tops and floors, sluices and bathrooms were frequent sources. In the present study also patient strains were recovered from many inanimate objects in the wards. (c.f. Table 3.34) Whitby and Rampling (245) made an interesting comparison of the level of pseudomonas contamination in hospital and the domestic milieu. Although only hospital staff were involved, pseudomonas was rarely observed in their private homes. None of the domestic strains by typing were identical to simultaneous isolates from the hospital.

There have been many previous reports of outbreaks of infection or cross-colonization by *P. aeruginosa* which have implicated items of equipment such as suction apparatus (246), resuscitation equipment (247, 248) and infant baths (249). Other workers have drawn attention to the risk from colonization of sinks (139), finding that sinks acted as a reservoir of pseudomonas which could be spread from patient to patient by contamination of staff hands during washing (250), and that patients or equipment within two metres of a sink contaminated with pseudomonas were more likely to acquire an infection or colonization with that strain than patients further from the sink. Other authors, however (134,251-253), have found that environmental strains of pseudomonas present in sinks and the like rarely cause infections in patients, although sinks may be transiently contaminated with strain that is causing infection in

patients.

In this hospital, the sinks are situated in very close proximity to the patients. Many of the sink swabs yielded a multiplicity of GRGNB. Of the 87 isolates of GRGNB from the sinks, 47 were *P. aeruginosa*, six were *P. cepacia*, four were *P. stutzeri* and the other 30 isolates were enterobacteria. The gentamicin-resistant pseudomonads and enterobacteria isolated from the sinks and other inanimate objects in the wards were similar to patient strains. All the isolates had the same degree of resistance (MIC) as the patient strains.

Antibiotic resistant organisms have been isolated in varying numbers from environmental studies conducted by other workers. Selden *et al* (121) showed that all isolates of multidrug resistant *Klebsiella* from the inanimate environmental study came from the patient areas. Specific objects positive for this organism included sink drains, floors, inhalation equipment, horizontal surfaces such as table tops and shelves, and a bathtub.

A survey by Noriega *et al* (130) of inanimate objects in use on the wards revealed resistant enteric bacilli only in empty urinals. Twelve of 23 contained gentamicin-resistant species.

Curie *et al* (77) showed that the immediate environment of colonized patients was often contaminated and settle plates placed near their beds frequently yielded colonies of gentamicin-resistant *K. aerogenes*. There was no evidence of persistance in the general ward environment and other settle plates were negative for the epidemic *Klebsiella*. The exceptions were certain sinks and drains which were frequently exposed to infected excreta. Contamination also persisted on a cotton floor-mop. Gentamicin-resistant

K. aerogenes was also recovered in small numbers from bedpans and urinals which had been inadequately disinfected.

In this study, 27 isolates of GRGNB were obtained from settle plates placed near the infected/colonized patients (c.f. Table 3.34). Gentamicin resistant gram negative bacilli were also recovered from unused bed pans (25 isolates) and urinals (23 isolates). These isolates were similar to patient strains.

Of 41 environmental samples taken by French *et al* (64), gentamicin resistant *Acinetobacter anitratus* was isolated from three stacked moist washing bowls and a kidney dish in the urological ward, dialysis fluid, ventilator tubing and a tracheal suction catheter from the intensive care ward.

Casewell *et al* (74) isolated gentamicin resistant *Klebsiella* from a bath, and from tooth mugs in a bathroom. Urinals, bedpans and washing machines were consistently negative in contrast to the study of Noriega cited above. The resistant organism was also isolated from four out of 25 samples from rooms in which colonized patients had been nursed.

Even pharmaceutical products have been incriminated. Ten per cent of 530 samples of medicaments were found to be contaminated with gram-negative bacteria in a study reported by Simon-Pujol *et al* (67). Multidrug-resistant *P. aeruginosa* was isolated from four solid and 15 liquid samples, of which 13 were oral medicaments and six were topical applications. Other gram-negative bacilli were isolated from 20 samples of oral medicaments and five from topical applications.

In this study four isolates of GRGNB were obtained from "in use" medicines administered orally to patients (c.f. Table 3.34) The results of this survey confirm studies in other countries that products in hospitals may become contaminated with micro-organisms during preparation or during use in the ward. If the bacteria are multiresistant, the implication of their presence in medicaments and food is even greater. There is no doubt that contaminated medicines and food can play a part in cross-infection and the preparation of these products and their storage should be rigidly controlled.

The hazards of contaminated anaesthesia and respiratory equipment have been amply documented (134,254). Respiratory infection associated with contaminated respiratory equipment was rarely reported between 1950 and 1965 (255,256), although Magath (257) had indicated the danger of contaminated anaesthesia machines and devised a water trap for filtering out bacteria in the expiratory tubing. The importance of high-humidity environments in the development of pseudomonas infections in newborn infants was also reported by Hoffmann and Finberg (258) and a marked degree of contamination in the water reservoir bottles of a humidifying device used in connection with oxygen therapy was described by Macpherson (259).

From 1965 onwards many reports have appeared which incriminate respiratory equipment in nosocomial respiratory infections mostly caused by gram-negative bacteria such as *Pseudomonas species*.

Phillips and Spencer (260) described an outbreak of cross-infection by *P. aeruginosa* in a general medical ward in which eight patients

became infected and two died. Swabbing and sampling showed that all the ventilators in use at the time were heavily contaminated with *P. aeruginosa*, especially the humidifer and expiratory tubing. The routine cleaning method did not eliminate the organisms. Ventilators in use and ventilators cleaned and ready for use on a new patient could still both pump *P. aeruginosa* directly into the patient's trachea, supporting their conclusion that contamination of the ventilators caused the outbreak. Phillips, in later studies (223,233) also employed pyocin and phage typing of pseudomonas strains, and by epidemiological means demonstrated machine-to patient spread.

Reinarz *et al* (261) also suggested an association between the increasing incidence of gram-negative necrotizing bacillary pneumonia and widespread use of inhalation therapy. They demonstrated that the nebulizer part of the equipment generated aerosols containing large numbers of viable bacteria.

In the nosocomial outbreaks later traced to respiratory equipment, the humidifying systems seemed to be the most vulnerable to bacterial contamination. Bacterial species involved are gram-negative bacilli such as *K. pneumoniae* (262), *P. aeruginosa* (167, 263), *P. cepacia* (111, 264), *P. fluorescens* (265) and *Acinetobacter anitratus* (71).

In this study 26 strains of GRGNB were isolated from the respiratory equipment used by patients on tracheostomy (c.f. TAble 3.34). The bacterial species isolated were *P. aeruginosa* (14 strains), *P.cepacia* (2 strains), *A. anitratus* (3 strains), *K. pneumoniae* (5 strains) and

Flavobacterium meningosepticum (2 strains).

Tracheal suction with contaminated catheters responsible for an outbreak of pseudomonas infection in two medical wards was described by Sutter *et al* (266), where phage typing was employed in the investigation. Direct inoculation of bacteria into the tracheobronchial tree occurred whether the patient was tracheostomized or was treated with suction through nasal or oral orifices.

Inhalation of contaminated aerosols can also occur in incubators (267) and humidification tents (268) with subsequent colonization of susceptible infants (269). Unheated room humidifiers were also demonstrated to be the major contaminating source in a *P. aeruginosa* outbreak where five of eight patients with pneumonia died (270).

Resuscitation equipment has also been responsible for outbreaks of neonatal *P. aeruginosa* infections either through contaminated electrical aspirators (247) or the oxygen mask, bag and tubing (126).

In the present study, ten isolates of GRGNB were obtained from suction apparatus and three from resuscitation equipment used in the intensive care unit (c.f. Table 3.34). The bacterial species involved were all *P. aeruginosa* which were similar to patient strains.

Equipment coming into direct contact with the upper respiratory tract is invariably contaminated with micro-organisms present in respiratory secretions (271). Contamination of the system with airborne or waterborne micro-organisms may also occur (272,273) and the moist environment provides suitable conditions for growth.

Epidemiological studies have shown that colonization of the airways with gram-negative bacilli is an important preceding event in the development of respiratory infections (275-278). Northey *et al*

(276) showed a colonization rate in throat and trachea of 55,8 per cent, and in the study of Johanson and co-workers (275) 45 per cent of the patients became colonized. Seventy-three per cent of the colonized patients developed pneumonia in comparison with 3,3 per cent of patients in whom colonization was not found.

Colonization is especially prominent in the presence of serious underlying illness (275,279), with endotracheal intubation (275) and in association with the use of antimicrobial drugs (275,279,280). The exact nature of the colonization phenomenon has been a matter of investigation and speculation during the last four years (281-283). The colonizing bacteria may be found in the oropharynx before localization in the airways and have either an endogenous (stomach or intestine) or an exogenous origin. Exogenous sources may be either human or inanimate objects.

In the present study, many of the patients with tracheostomy had positive cultures for GRGNB from more than one site and this included the oropharynx region.

Respiratory equipment undoubtedly acts as an infection reservoir and constitute a hazard to susceptible patients. A rigorous programme for decontamination is therefore recommended and has been shown to be effective in prevention of equipment-associated epidemics of respiratory infections (247,274,285).

The results obtained from the environmental surveillance in this study (c.f. Table 3.34) support the work done by others (67,77,83, 121), in clearly demonstrating the environment as a source of GRGNB.

In this study there was clear evidence of cross infection and colonization between patients, fomites and staff members. The various GRGNB found in clinical specimens from patients was also found on inanimate environment and on staff members. It cannot be established for certain whether the staff members or the fomites acted as routes of transmission.

CONCLUSION

The findings of this study confirm that *P. aeruginosa* and *K. pneumoniae* remain important causes of cross-infection particularly in the intensive care setting. I have further established that a particular pyocin, phage and sero type of *P. aeruginosa* is responsible for the vast majority of pseudomonas infections. All the isolates in this study were also found to be resistant to gentamicin and to many of the other antitibiotics tested. It is therefore obvious that to curb the increase of antibiotic-resistance among the hospital flora, one needs to have a strict antibiotic policy and also rigid guidelines for the prevention of cross-infection. Fortunately there are antibiotics now available to treat gentamicin-resistant gram-negative bacterial infections. These include the third generation cephalosporins and piperacillin. However, numerous outbreaks that have been cited in this, and in other studies have clearly implicated the role of hands as a predominant source of cross-infection. Therefore, one needs to reinforce the simple technique of hand washing, rather than resorting to potent antibiotics to control the increasing incidence of hospital acquired infections.

Hand washing before and after contact with each patient remains the single most important means of preventing the spread of infections.

REFERENCES

1. Editorial (1966). Infectious drug resistance. New Eng. J. Med. 275 : 277

2. Garrod, L.P., Lambert, H.P. and O"Grady, F. (1973). Antibiotic and chemotherapy, 4th ed., Livingstone, Edinburgh, pp 1-11

3. Aoki, T., Egusa, S., Ogata, Y. and Watanabe, T. (1971). Detection of resistance factors in fish pathogen *Aeromonas liquefaciens*. J. Gen. Microbial. 65 : 343-349

4. Jukes, J. (1971). The present status and background of antibiotics in the breeding of domestic animals. Annals of the New York Academy of Sciences 182 : 362-379

5. Isenberg, H. and Berkman, J. (1971). The role of drug-resistant and drug-selected bacteria in nosocomial disease. Annals of the New York Academy of Science 182 : 52-58

6. Buckwold, F.J. and Ronald, A.R. (1979). Antimicrobial misuses - effects and suggestions for control. J. Antimicrob. Ag. Chemo. 5 : 129-136

7. Bryan, L.E. (1982). Current problems of resistant bacteria. In : Bacterial resistance and susceptibility to chemotherapeutic agents. pp 161-191 Cambridge University Press, Cambridge

8. Davies, J. (1971). Bacterial resistance to aminoglycoside antibiotics. J. Inf. Dis., Suppl 124 : 7

9. Tseng, J.T., Bryan, L.E. and Van Den Elzen, H.M., (1972). Mechanisms and spectrum of streptomycin resistance in a natural population of *P. aeruginosa*. Antimicrob. Ag. Chemo. 2 : 136-141

10. Abraham, E.P. and Chain, E. (1940). An enzyme from bacteria able to destroy penicillin. Nature, Lond 146 : 837

11. Hayes, W. (1968). The Genetics of bacteria and their viruses, 2nd ed., pp 179-223. Blackwell, Oxford.

12. Davies, J.E. and Rownd, R. (1972). Transmissible multiple drug resistance in enterobacteriaceae. Science, New York 176 : 758-768

13. Richmond, M.H. (1972). Some environmental consequences of the use of antibiotics : or "What goes up must come down". J. Appl. Bact. 35 : 155-176

14. Jawetz, E. (1963). Antibiotics revisited : problems and prospects after two decades. Br. Med. J. 3 : 951-955

15. Munch-Peterson, E. and Boundy, C. (1962). Yearly incidence of penicillin resistant staphylococci in man since 1942. Bull WHO 26 : 241-252

16. Anderson, E.S. (1968). The ecology of transferable drug resistance in the enterobacteria. Ann. Rev. Microbiol. 22 : 131-180

17. Novick, R.P., Clowes, R., Cohen, S., Curtiss III, R., Datta, N. and Falkow, S. (1976). Uniform nomenclature for bacterial plasmids. Bacteriological Reviews 40 (1) : 168-189

18. Clowes, R.C. (1972). Molecular structure of bacterial plasmids. Bact. Rev. 36 : 361-405.

19. Watanable, J. (1971). Infectious drug resistance in bacteria. Curr. Top. Microbial. Immunol. 56 : 43-98

20. Akiba, T. (1959). Mechanisms of the development of drug resistance in Shigella. Proc. of the 15th General Assembly of Japan Med. Assoc. 5 : 299-305

21. Watanabe, T. (1963). Infective heredity of multiple drug resistance in bacteria. Bact. Rev. 27 : 87-115

22. Maré, I.J. and Coetzee, J.N. (1965). The isolation of Enterobacteriaceae possessing the property of transmissible multiple drug resistance. S.A. Med J. 39 : 864

23. Falkow, S. (1975). Infectious multiple drug resistance. Pion Ltd., London

24. Datta, N. and Hedges, R. (1972). Trimethoprim resistance conferred by R- plasmids in Enterobacteriaceae. J. Gen. Micro. 72 : 349-356

25. Chabbert, Y. and Witchitz, J. (1972). *In vivo* transfer of a plasmid governing gentamicin resistance. In : Bacterial plasmids and antibiotic resistance, ed. Kremery, V., Rosival, L. and Watanabe, T. Springer-Verlag, Berlin

26. Datta, N. (1965). Infectious drug resistance. Br. Med. Bull. 21 : 254-259

27. Mikhael, I.A., Kent, D.C., Soernsen, K., Sanborn, W.R. and Smith, J. (1972). Concentrations of ampicillin and chloramphenicol in the serum of patients with acute Salmonella enteric fever. Antim. Agents Chemo. 2 : 336-339

28. Hammel, R.P., Miskell, P.W. and Altemeier, W.A. (1977). Antibiotic resistance transfer from non-pathogenic to pathogenic bacteria. Surgery 82 : 382-385

29. Kimura, S., Ikeda, T., Eda, T., Mitsui, Y. and Nakata, K. (1976). R-plasmids from Yersinia. J. Gen. Microbiol. 97 : 141-144

30. Datta, N. and Hedges, R.M. (1972). Host ranges of R-factors. J. Gen. Microbiol. 70 : 453-460.

31. Bryan, L.E. (1982). Mechanisms of resistance to antibacterial agents. In : Bacterial resistance and susceptibility to chemotherapeutic agents. pp 69-103 Cambridge University Press, Cambridge

32. Nakahara, H., Ishikawa T., Sarai, Y., Kondo, I. and Mitsuhashi, S. (1977). Frequency of heavy-metal resistance in bacteria from in-patients in Japan. Nature 66 : 165-167

33. Datta, N. (1969). Drug resistance and R factors in the bowel bacteria of London patients before and after admission to hospital. B.M.J. 2 : 407-411

34. Moorhouse, E.C. (1969). Transferable drug resistance in enterobacteria isolated from urban infants. B.M.J. 2 : 405-407

35. Linton, K., Richmond, M., Bevan, R. and Gillespie (1974). Antibiotic resistances and R-factors in coliform bacilli isolated from hospital and domestic sewage. J. Med. Microbiol. 7 : 91-103

36. Aronson, A.L. (1975). The use, misuse and abuse of antibacterial agents. Modern Veterinary Practice 56(6) : 383-389

37. Guinee, P.A.M. (1971). Bacterial drug resistance in animals. Annals of the New York Academy of Sciences 182 : 40-51

38. Kremery, V., Rosival, L., Vymola, F. and Hejzlar, M. (1972). Problems of transferable antibiotic resistance in Czechoslovakia. In : Bacterial plasmids and antibiotic resistance ed. Kremery, V., Rosival, L. and Watanabe T. Springer-Verlag Berlin

39. Lebek, G. (1972). Epiedmilogical investigations of R factors in man and animals in Switzerland. In : Bacterial plasmids and antibiotic resistance ed. Kremery, V., Rosival, L. and Watanabe, T. Springer-Verlag Berlin

40. Gardner, P., Smith, D.H., Beer, H. and Moellering, R.C. Jr. (1969) Recovery of resistance (R) factors from a drug-free community. Lancet 2 : 774-776

41. Mare, I.J. (1968). Incidence of R factors among gram negative bacteria in drug-free human and animal communities. Nature 220 : 1046-1047

42. Smith, H.W. (1967). The effect of the use of antibacterial drugs particularly as food additives, on the emergence of drug resistant strains of bacteria in animals. New Zealand Veterinary Journal 15(9): 153-166

43. Benveniste, R. and Davies, J. (1973). Mechanisms of antibiotic resistance in bacteria. Ann. Rev. Biochem. 42 : 471-506

44. Phillips, I. and Shannon, K. (1984). Antibiotic resistance in bacteria. British Medical Bulletin 40 (1) : 29-35

45. Linton, A.H. (1977). Antibiotic resistance : the present situation reviewed. Veterinary Record 100 : 354-360

46. Kelly, W.R., Moorhouse, E. and Collins, J. (1971). The incidence and importance of antibiotic resistant bacteria in livestock in the Republic of Ireland. J. Irish Med. Assoc. 62 (404) : 29-35

47. Walton, J.R. (1972). Antibiotics, animals and R factors in man. In : Bacterial plasmids and antibiotic resistance. ed. Kremery, V., Rosival, L. and Watanabe, T. Springer-Verlag Berlin

48. Mercer, H.D. (1975). Antimicrobial drugs in food producing animals : control mechanisms of governmental agencies. Vet. Clin. North America 5 (1) : 3-34

49. Swann, M. (1969). Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine. Her Majesty's Stationery Office, London

50. Forbes, I., Gray, A., Hurse, A. and Pavillard, R. (1977). The emergence of gentamicin resistant *Klebsiellae* in a large general hospital. Med. J. Australia 1 : 14-16

51. Richmond, A., Simberkoff, M., Raha Jr. J. and Schaefler, S. (1975). R factors in gentamicin resistance organisms causing hospital infections. Lancet 2 (1946) : 1176-1178

52. Holmes, R.K., Minshew, B.H., Gould, K., Sanford, J. (1974). Resistance of *Pseudomonas aeruginosa* to gentamicin and related aminoglycoside antibiotics. Antmicrob. Agents Chemo. 6 : 253-262

53. Chadwick, P. (1973). Resistance of *Pseudomonas aeruginosa* to gentamicin. Can. Med. Assoc. Jnl. 109 : 585-587

54. Shulman, J.A., Terry, P.M. and Hough, C.E. (1971). Colonization with gentamicin resistant *Pseudomonas aeruginosa*, pyocine type 5, in a burn unit. J. Infect. Dis. 124 (suppl) : 518-523

55. Bryan, L.E., Shahrabadi, M.S. and Van Den Elzen, H.M. (1974). Gentamicin resistance in *Pseudomonas aeruginosa* : R factor mediated resistance. Antimicrob. Agents Chemo. 6 : 191-199

56. Washington, J.A., Senjem, D.H., Haldorson, A., Schutt, A.H. and Martin, W.J. (1973). Nosocomially acquired bacteriuria due to *Proteus rettgeri* and *Providencia stuartii*. J. Clin. Pathol. 60 : 836-838

57. Edwards, L.D., Cross, A., Levin, S. and Landau, W. (1974). Outbreak of a nosocomial infection with a strain of *Proteus rettgeri* resistant to many antimicrobials. Am. J. Clin. Pathol. 61 : 41-46

58. Overturf, G.D., Wilkins, J. and Resaler, R. (1974). Emergence of resistance of *Providencia stuartii* to multiple antibiotics : speciation and biochemical characterization of Providencia. J. Infect. Dis. 129 : 353-357

59. Martin, C.M., Ikari, N.S., Zimmerman, J. and Waitz, J.A. (1971). A virulent nosocomial *Klebsiella* with a transferable R factor for gentamicin: emergence and suppression. J. Infect. Dis. 124 (Suppl.):S24-S29.

60. Finland, M. (1972). Changing patterns of susceptibility of common bacterial pathogens to antimicrobial agents. Ann. Intern. Med. 76 : 1009-1036.

61. Platt, D.J., and Somerville, J.S. (1981). *Serratia species* isolated from patients in a general hospital. Jnl. Hosp. Infect. 2 : 341-348

62. Alvarez, J.S., and Regueiro, R. (1980). R plasmid from clincial isolates of *Serratia marcescens*. Jnl. Hosp. Infect. 1 : 133-139

63. Perinpanayagam, R.M. and Grundy, H.C. (1983). Outbreak of gentamicin resistant *Pseudomonas aeruginosa* infection in a burns unit. Jnl Hosp. Infect. 4 : 71-73

64. French, G.L., Casewell, M.W., Roncoroni, A.J., Knight, S. and Phillips, I. (1980). A hospital outbreak of antibiotic resistant *Acinetobacter anitratus* : epidemiology and control. Jnl. Hosp. Infect. 1 : 125-131

65. Holton, J. (1982). A report of a further hospital outbreak caused by a multi-resistant *Acinetobacter anitratus*. Jnl. Hosp. Infect. 3 : 305-309

66. Bullock, D.W. Bidwell, J.L., Reeves, D.S., White, L.O., Turner, A., Speller, D.C.E. and Wilkinsons (1982). Outbreaks of hospital infection in southwest England caused by gentamicin resistant *Serratia marcescens*. Jnl. Hosp. Infect. 3 : 263-273

67. Simon-Pujol, M.D., Matabosch, M., Espuny-Tomas, M.J., Marques, A.M., and Congregado, F. (1981). Drug resistance patterns of Gram negative bacteria isolated from food and non-sterile medicines in hospital environments. Jnl. of Hosp. Infect. 2 : 355-361

68. Mutton, K.J., Brady, L.M. and Harkness, J.L. (1981). *Serratia* cross-infection in an intensive therapy unit. Jnl. Hosp. Infect. 2 : 85-91

69. Smith, P.W. and Rusnak, P.G. (1981). Aminoglycoside resistant *Pseudomonas aeruginosa* urinary tract infection : study of an outbreak. Jnl. Hosp. Infect. 2 : 71-75

70. Blenkharn, J.I. and Hughes, V.M. (1982). Suction apparatus and hospital infection due to multiply resistant *Klebsiella aerogenes*. Jnl. Hosp. Infect. 3 : 173-178

71. Buxton, A.F., Anderson, R.L., Werdegar, D. and Atlas, E. (1978). Nosocomial respiratory tract infection and colonization with *Acinetobacter calcoaceticus*. Amer. J. Med. 65 : 507-513

72. Schaberg, D.R., Alford, R.H., Anderson R., Farmer III, J.J., Melly, M.A. and Schaffner, W. (1976). An outbreak of nosocomial infection due to multiply resistant *Serratia marcescens* : Evidence of interhospital spread. Jnl. Infect. Dis. 134 : 181-188.

73. Meyer, R.D., Lewis, R.P., Halter, J. and White, M. (1976). Gentamicin resistant *Pseudomonas aeruginosa* and *Serratia marcescens* in a general hospital. Lancet 1 : 580-583

74. Casewell, M.W. and Phillips, I. (1977). Hands as a route of transmission for *Klebsiella species*. B.M.J. 2 : 1315-1317

75. Casewell, M.W. and Phillips,J.(1978). Food as a source of *Klebsiella species* for colonization and infection of intensive care patients. J. Clin. Path. 31 : 845-849

76. Gordon, A.M. (1980). Gentamicin resistant *Klebsiella* strains in a hospital. B.M.J. 1 : 722-723

77. Curie, K., Speller, D.C.E., Simpson, R.A., Stephens, M. and Cooke, D.I. (1978). A hospital epidemic caused by a gentamicin resistant *Klebsiella aerogenes*. Jnl. Hyg., Cambridge 80 : 115-123

78. Houang, E.T., Casewell, M.W., Simms, P.A. and Horton, R.A. (1980). Hospital acquired faecal *Klebsiella* as source of multiple resistance in the community. Lancet 1 : 148-149

79. Schaberg, D.R., Weinstein, R.A. and Stamm, W.E. (1976). Epidemics of nosocomial urinary tract infection caused by multiply resistant gram negative bacilli : epidemiology and control. J. Infect. Dis. 133 : 363-366

80. Thomas, F.E., Jackson, R.T., Melly, A. and Alford, R.H. (1977). Sequential hospital-wide outbreaks of resistant *Serratia* and *Klebsiella* infections. Arch. Int. Med. 137 : 581-584

81. Hughes, V.M., Datta, N. and Faiers, M.C. (1981). Interhospital spread of a multiply resistant *Klebsiella*. B.M.J. 282 : 696

82. Gerding, D.N., Buxton, A.E., Hughes, R.A., Cleary, P.P., Abraczawski, J. and Stamm, W.E. (1979). Nosocomial multiply resistant *Klebsiella pneumoniae* : epidemiology of an outbreak of apparent index case origin. Antimicrob. Ag. & Chemo. 15 : 608-615

83. Hart, C.A. (1982). Nosocomial gentamicin and multiply resistant enterobacteria at one hospital. 1. Description of an outbreak. Jnl. Hosp. Infect. 3 : 15-28

84. Houang, E.T., Evans, M.A.L. and Simpson, C.N. (1979). Control of hospital epidemic of gentamicin resistant *Klebsiella aerogenes*. Lancet 2 : 205.

85. Noy, J.H., Ayliffe, G.A.J. and Linton, K.B. (1974). Antibiotic resistant gram negative bacilli in the faeces of neonates. Jnl. Med. Micro. 7 : 509-520

86. Gaman, W., Cates, C., Snelling, C.F.T., Lank, B. and Ronald, A.R. (1976). Emergence of gentamicin and carbenicillin resistant *Pseudomonas aeruginosa* in a hospital environment. Antimicrob. Ag. & Chemo. 9 : 474-480

87. Fujita, K., Lilly, H.A. and Ayliffe, G.A.J. (1982). Spread of resistant gram negative bacillli in a burns unit. Jnl. Hosp. Infect. 3 : 29-37

88. Block, C.S. (1978). Gentamicin resistant gram negative bacilli in hospital patients. 1. Preliminary epidemiological assessment. S.A.M.J. 53 : 391-395

89. Block, C.S., Fabian, B. and Robinson, R.G. (1978). Gentamicin-resistant gram-negative bacilli in hospital patients. II. *In vitro* aspects including susceptibility to tobramycin and amikacin. S.A.M.J. 53 : 396-399.

90. Casewell, M.W. and Talsania, H.G. (1981). Multiple antibiotic resistance and capsular types of gentamicin resistant *Klebsiella aerogenes*. Jnl. Antimicrob. Chemo. 7 : 237-244

91. Leonard, J.M., McGee, Z.A. and Alford, R.H. (1978). Gentamicin resistant bacilli. Infect. Arch. Intern. Med. 138 : 201-205

92. Holloway, B.W. (1960). Grouping *Pseudomonas aeruginosa* by lysogenicity and pyocinogenicity. Jnl. Path. Bact. 80 : 448-450.

93. Henriksen, S.D., (1973). *Moraxella, Acinetobacter,* and the Mimeae . Bacteriological Reviews 37 : 522-561

94. Al-Khoja, M.S. and Darrell, J.H. (1979). The skin as a source of *Acinetobacter* and *Moraxella* species occuring in blood cultures. Jnl. Clin. Path. 32 : 497-499

95. Buxton, A.E., Anderson, R.L., Werdegar, D. and Atlas, E. (1978). Nosocomial respiratory tract infection and colonization with *Acinetobacter calcoaceticus*. Epidemiologic characteristics. Jnl. Amer. Med. Assoc. 65 : 507-513

96. Glew, R.H., Moellering, R.C. and Kunz, L.J. (1977). Infections with *Acinetobacter calcoaceticus* (Herellea vaginicola) : clinical and laboratory studies. Medicine 56 : 79-97

97. Ramphael, R. and Kluge, R.M. (1979). *Acinetobacter calcoaceticus* variety *anitratus* : an increasing nosocomial problem. Amer. Jnl. Med. Sc. 277 : 57-66

98. Rosenthal, S.L. and Freundlich, L.F. (1977). The clinical significance of *Acinetobacter species*. Health Laboratory Science 14 : 194-198

99. Richmond, M.H. (1973). Resistance factors and their ecological importance to bacteria and man. Progress in Nucleic Acid Research and Molecular Biology 13 : 191-248

100. Burkholder, W.H. (1950). Sour skin, a bacterial rot of onion bulbs. Phytopathology 40 : 115-117

101. Ballard, R.W., Palleroni, N.J., Doudoroff, M., Stanier, R.Y. and Mandel, M. (1970). Taxonomy of the aerobic Pseudomonads : *P. cepacia, P. marginata, P. alliicola* and *P. caryophilli*. Jnl. Gen. Micro. 60 : 199-214

102. Taplin, D., Bassett, D.C.J. and Mertz, P.M. (1971). Foot lesions associated with *Pseudomonas cepacia*. Lancet 2 : 568-571

103. Noriega, E.R., Rubinstein, E., Simberkoff, M.S. and Rahal, J.J. (1975) Subacute and acute endocarditis due to *Pseudomonas cepacia* in heroin addicts. Amer. Jnl. Med. 59 : 29-36

104. Ederer, G.M. and Matsen, J.M. (1972). Colonization and infection with *Pseudomonas cepacia*. Jnl.Infect. Diseases. 125 : 613-618

105. Rosenstein, B.J. and Hall, D.E. (1980). Pneumonia and septicaemia due to *Pseudomonas capacia* in a patient with cystic fibrosis. John Hopkins Medical Journal 147 : 188-189

106. Speller, D.C.E., Stephens, M.E. and Viant, A.C. (1971). Hospital infection by *Pseudomonas cepacia*. Lancet 1 : 798-799

107. Rahal, J.J., Simberkoff, M.S. and Hyams, P.J. (1973). *Pseudomonas cepacia* tricuspid endocarditis : treatment with trimethoprim sulphonamide and polymyxin B. Jnl. Infect. Dis. 128 (suppl) 762-767

108. Kothari, T., Reyes, M.P., Brooks, N., Brown, W.J. and Lerner, A.M. (1977). *Pseudomonas cepacia* septic arthritis due to intra-articular injections of methylprednisone. Canadian Medical Assoc. Jnl. 116 : 1230-1232

109. Berkelman, R.L., Godley, J., Weber, J.A., Anderson, R.L., Lerner, A.M., Paterson, H.J. and Allen, J.R. (1982). *Pseudomonas cepacia* peritonitis associated with contamination of automatic peritoneal dialysis machines. Annals of Internal Med. 96 : 456-458

110. Martone, W.J., Osterman, C.A., Fisher, K.A. and Wenzel, R.P. (1981). *Pseudomonas cepacia* : Implications and control of epidemic nosocomial colonization. Reviews of Infectious Diseases 3 : 708-715

111. Schaffner, W., Reisig, G. and Verrall, R.A. (1973). Outbreak of *Pseudomonas cepacia* infection due to contaminated anaesthetics. Lancet 1 : 1050-1051

112. Weinstein, R.A., Emori, T.G., Anderson, R.L. and Stamm, W.E. (1976). Pressure transducers as a source of bacteremia after open heart surgery - report of an outbreak and guidelines for prevention. Chest 69 : 338-344

113. Guiness, M. and Levey, J. (1976). Contamination of aqueous dilutions of Resiguard disinfectant with *Pseudomonas*. Med. Jnl. Australia 2 : 392

114. Craven, D.E., Moody, B., Connolly, M.G., Kollisch, N.R., Stottmeier, K.D. and McCabe, W.R. (1981). Pseudobacteremia caused by Providone-Iodine solution contaminated with *Pseudomonas cepacia*. New England Jnl. Med. 305 : 621-623

115. Pappalardo, G., Roussionos, D. and Tanner, F. (1980). Hospital infections by *Pseudomonas cepacia*. Experientia 36 : 493

116. Carson, L.A., Favero, M.S., Bond, W.W. and Peterson, N.J. (1973). Morphological, biochemical and growth characteristics of *Pseudomonas cepacia* from distilled water. Applied Microbiology 25 : 476-483

117. Dixon, R.E., Kaslow, R.A., Mackel, D.C., Fulkerson, C.C. and Mallison, G.F. (1976). Aqueous quaternary ammonium antiseptics and disinfectants - use and misuse. Jnl. Amer. Med. Assoc. 236 : 2415-2417

118. Gonzalez, C.F. and Vidaver, A.K. (1979). Bacteriocin, plasmid and pectolytic diversity in *Pseudomonas cepacia* of clinical and plant origin. Jnl. Microbiology 110 : 161-170

119. Gilardi, G.L. (1971). Characterization of *Pseudomonas species* isolated from clinical specimens. Appl. Micro. 21 : 414-419

120. Moody, M.R., Young, V.M. and Kenton, D.M. (1972). *In vitro* antibiotic susceptibility of pseudomonads other than *Pseudomonas aeruginosa* recovered from cancer patients. Antimicrob. Ag. & Chemo. 2 : 344-349

121. Selden, R., Lee, S., Wang, W.L.L., Bennett, J.V. and Eickhoff, T.C. (1971). Nosocomial *Klebsiella* infections : intestinal colonization as a reservoir. Annals Int. Med. 74 : 657-664

122. Hart, C.A., Gibson, M.F. and Buckles, A. (1981). Variation in skin and environmental survival of hospital gentamicin resistant enterobacteria. Jnl. Hyg., Cambridge 87 : 277-285

123. Guerrant, R.L., Strausbaugh, L.J., Wenzel, R.P., Hamory, B.H. and Sande, M.A. (1977). Nosocomial bloodstream infections caused by gentamicin resistant gram negative bacilli. The Amer. Jnl. Med. 62 : 894-901

124. Yu, V. (1979). *Serratia marcescens*, historical perspective and clinical review. New Eng. Jnl. Med. 300 : 887-893

125. Casewell, M.W., Dalton, M.T., Webster, M. and Phillips, I. (1977). Gentamicin resistant *Klebsiella aerogenes* in a urological ward. Lancet 2 : 444-446

126. Falkiner, F.R., Keane, C.F., Dalton, M., Clancy, M.T. and Jacoby, G.A. (1977). Cross infection in a surgical ward caused by *Pseudomonas aeruginosa* with transferable resistance to gentamicin and tobramycin. J. Clin. Path. 30 : 731-737

127. Bulger, R.J., Roberts, C.F. and Sherris, J.C. (1967). Changing incidence of antibiotic resistance among *Staphylococcus aureus*, *Escherichia coli*, *Aerobacter Klebsiella*, and *Pseudomonas* encountered in a teaching hospital over a 7-year period. Antimicrob. Ag. & Chemo. 1966 : 42-46

128. Ridley, M., Barrie, D., Lynn, R. and Stead, K.C. (1970). Antibiotic resistant *Staphylococcus aureus* and hospital antibiotic policies. Lancet 1 : 230-233

129. Price, D.J.E. and Sleigh, J.D. (1970). Control of infection due to *Klebsiella aerogenes* in a neurosurgical unit by withdrawal of all antibiotics. Lancet 2 : 1213-1215

130. Noriega, E.R., Leibowitz, R.E., Richmond, A.S., Rubinstein, E., Schaefler, S., Simberkoff, M.S. and Rahal, J.J. (1975). Nosocomial infection caused by gentamicin resistant, streptomycin sensitive *Klebsiella*. J. Infect. Dis. 131 (suppl) : S45-S50

131. Muller, F.E. (1967). Gentamicin in the treatment of *Pseudomonas* infections complicating burns. Proceedings of Gentamicin-First International Symposium, Paris

132. Snelling, C.F.T., Ronald, A.R., Kernahan, D.A., Waters, W.R. and Vistnes, L.M. (1970). Topical gentamicin in burns. Antimicrob. Ag. Chemo. 1969 : 380-385

133. Keys, T.F. and Washington II, J.A. (1977). Gentamicin resistant *Pseudomonas aeruginosa*. Mayo Clinic Experience, 1970-1976. Mayo Clin. Proc. 52 : 797-801

134. Lowbury, E.J.L., Thom, B.T., Lilly, H.A., Babb, J.R., and Whittal, K. (1970). Sources of infection with *Pseudomonas aeruginosa* in patients with tracheostomy. J. Med. Microbiol. 3 : 39-56

135. Tomioka, S., Kobayashi, Y. and Hasegawa, M. (1976). Proceedings of the 9th International Congress of Chemotherapy.

136. Greene, W.H., Moody, S., Schimpff, S., Young, V.M. and Wiernick, P.H. (1973). *Pseudomonas aeruginosa* resistant to carbenicillin and gentamicin. Ann. Intern. Med. 79 : 684-689

137. Knothe, H., Kiemery, V., Sietzen, W. and Barst, J. (1973). Transfer of gentamicin resistance from *Pseudomonas aeruginosa* strains highly resistant to gentamicin and carbenicillin. Chemotherapy 18 : 229-234

138. Gaya, H. (1974). The bacteriology of intensive care. Brt. Jnl. Hosp. Med. 2 : 853-859

139. Teres, D., Schweers, P., Bushnell, L.S., Hedley-White, J. and Feingold, D. (1973). Sources of *Pseudomonas aeruginosa* infection in a respiratory/surgical intensive therapy unit. Lancet 1 : 415-417

140. Stokes, E.J. and Waterworth, P.M. (1972). Antibiotic sensitivity tests by diffusion methods. Assoc. Clin. Path. Broadsheet 55 : 1-12

141. Wahba, A.H. and Darrell, J.H. (1965). The identification of atypical strains of *Pseudomonas aeruginosa*. Jnrl. Gen. Microb. 38 : 329-342

142. Phillips, I. (1969). Identification of *Pseudomonas aeruginosa* in the clinical laboratory. Jnl. Med. Micro. 2 : 9-16

143. King, A. and Phillips, I. (1978). The identification of Pseudomonads and related bacteria in a clinical laboratory. Jnl. Med. Micro. 2 : 165-176

144. Cowan, S.T. and Steel, K.H. (1974). Manual for the Identification of Medical Bacteria. Cambridge University Press, London.

145. Edwards, P.R. and Ewing, W.H. (1972). Identification of *Enterobacteriaceae*. Burgess Publishing Company, Minnesota

146. MacFaddin, J.F. (1980). Biochemical Tests for Identification of Medical Bacteria. Baltimore, Williams and Wilkins.

147. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardised single disc method. Amer. Jnl. Clin. Path. 45 : 493-496

148. Ericsson, H. and Sherris, J.C. (1971). Antibiotic sensitivity testing. Report of an International Collaborative Study. Acta Pathologica et Microbiologica Scandinavica, Section B. Supplement number 217

149. Ferderal Register (1972). Vol. 37, No. 191, Sept. 30

150. National Committee for Clinical Laboratory Standards Subcommittee on antimicrobial susceptibility testing (1975). Performance standards for antimicrobial disc susceptibility tests. Approved Standard : A5M-2

151. Gillies, R.R. and Govan, J.R.W. (1966). Typing of *Pseudomonas pyocyanea* by pyocine production. J. Path. Bact. 91 : 339-345

152. Govan, J.R.W. and Gillies, R.R. (1969). Further studies in the pyocine typing of *Pseudomonas pyocyanea*. J. Med. Microbiol. 2 : 17-25

153. Govan, J.R.W. (1978). Pyocin typing of *Pseudomonas aeruginosa*. In : Methods in microbiology. Vol. 10. Academic Press, London. pp 61-69

154. Gratia, A. (1947). Technique de la 'double-couche'. Bull. Soc. Chim. biol. 29 : 352

155. Peterson, Ann C. (1965). Bacteriocinogeny and lysogeny in the genus *Pseudomonas*. J. Gen. Microbiol. 39 : 295

156. Farmer, J.J. and Herman, L.G. (1969). Epidemiological fingerprinting of *Pseudomonas aeruginosa* by the production of and sensitivity to pyocin and bacteriophage. Appl. Microbiol. 18 : 760

157. Rampling, Anita and Whitby, J.L. (1972). Preparation of phage-free pyocin extracts for use in the typing of *Pseudomonas aeruginosa*. J. Med. Microbiol. 5 : 305-312

158. Rampling, A., Whitby, J.L. and Wildy, P. (1975). Pyocin sensitivity testing as a method of typing *Pseudomonas aeruginosa*. Use of 'phage-free' preparations of pyocin. Jnl. Med. Microbiology 8 : 531-541.

159. Adams, M.H. (1959). In:bacteriophages. Appendix : Methods of study of bacterial viruses. New York, Interscience Publishers, Inc.

160. Coetzee, J.N. and Sacks, T.G. (1960). Morphological variants of *Proteus hauseri*. J. of Gen. Microb. 23 : 209

161. Govan, J.R.W. (1978). Pyocin typing of *Pseudomonas aeruginosa*. In : Methods in Microbiology. ed. T. Bergan and J.R. Norris. Vol.10 Academic Press, London, pp 61-91.

162. Siem, T.H. (1972). Het typeren van *Pseudomonas aeruginosa* met behulp van een gecombineerde sero-pyocine typeringsmethode. Doctorate thesis presented in the University of Amsterdam.

163. Heckman, M.G., Babcock, J.B. and Rose,H.D. (1972). Pyocine typing of *Pseudomonas aeruginosa* : clinical and epidemiologic aspects. Amer. Jnl. Clin. Path. 57 : 35-42

164. Deighton, M.A., Tagg, J.R. and Mushin, R. (1971). Epidemiology of *Psuedomonas aeruginosa* infection in hospitals. Med. Jnl. Australia 1 : 892-896

165. Govan, J.R.W. (1968). The pyocines of *Pseudomonas pyocyanea*. Thesis presented for the Degree of Doctor of Philosophy, University of Edinburgh

166. Duncan, I.B.R. and Booth, E.V. (1975). Epidemiology of *Pseudomonas aeruginosa* infections investigated by pyocin typing. Canadian Medical Assoc. Jnl. 112 : 837-843

167. Tinne, J.E., Gordon, A.M., Bain, W.H. and Mackey, W.A. (1967). Cross-infection by *Pseudomonas aeruginosa* as a hazard of intensive surgery. B.M.J. 4 : 313-315

168. Shooter,R.A., Walker, K.A., Williams, V.R., Horgan, G.M., Parker, M.T., Asheshov, E.H. and Bullimore, J.F. (1966). Faecal carriage of *Pseudomonas aeruginosa* in hospital patients. Possible spread from patient to patient. Lancet 2 : 1331-1334

169. Meitert, T. and Meitert, E. (1978). Epidemiological typing methods. In : Methods in Microbiology. ed. T. Bergan and J.R. Norris. Vol.10 Academic Press. London, pp 1-17

170. Lanyi, B. and Bergan, T. (1978). Serological characterization of *Pseudomonas aeruginosa*. In : Methods in Microbiology. ed. T. Bergan and J. Norris. Vol. 10. Academic Press. London, pp 93-168

171. Sato, H. and Diena, B.B. (1974). Serological survey of *Pseudomonas aeruginosa* strains isolated from patients in Canadian hospitals. Canadian Jnl. of Micro. 20 : 477-482

172. Al-Dujaili, A.H. and Harris, D.M. (1974). Evaluation of commercially available antisera for serotyping of *Pseudomonas aeruginosa*. Jnl. Clin. Path. 27 : 569-571

173. Knights, H.T., France, D.R. and Harding, S. (1968). *Pseudomonas aeruginosa* cross infection in a neonatal unit. New Zealand Med. Jnl. 67 : 617-620

174. Ayliffe, G.A.J., Lowbury, E.J.L., Hamilton J.G., Small, J.M., Asheshov, E.A. and Parker, M.T. (1965). Hospital infection with *Pseudomonas aeruginosa* in neurosurgery. Lancet 2 : 365-369

175. Ayliffe, G.A.J., Barry, D.R., Lowbury, E.J.L., Roper-Hall, M.J., and Walker, W.M. (1966). Postoperative infection with *Pseudomonas aeruginosa* in an eye hospital. Lancet 1 : 1113-1117

176. Waterworth, P.M. (1972). The *in vitro* activity of tobramycin compared with that of other aminoglycosides. Jnl. Clin. Path. 25 : 979-983

177. Waitz, J.A., Moss, E.L., Drube, C.G. and Weinstein, M.J. (1972). Comparative activity of sisomicin, gentamicin, kanamycin and tobramycin. Antimicrob. Ag. & Chemo. 2 : 431-436

178. Klastersky, J., Henri, A., Hensgens, C., Vandenborre, L. and Daneau, D. (1973). Antipseudomonal drugs : Comparative study of gentamicin, sisomicin and tobramycin *in vitro* and in human volunteers. European Jnl. of Cancer 9 : 641-648

179. Reynolds, A.V., Hamilton-Miller, J.M.T. and Brumfitt, W. (1974). Newer aminoglycosides - amikacin and tobramycin : an *in vitro* comparison with kanamycin and gentamicin. B.M.J. 3 : 778-780

180. Mayer, R.D., Lewis, R.P., Carmalt, E.D. and Finegold, S.M. (1976). Amikacin therapy for serious gram negative bacilliary infections. Annals of Inter. Med. 83 : 790-793

181. Shah, P.M., Heetderks, G. and Stille, W. (1977). Bactericidal activity of amikacin and gentamicin. Chemotherapy 23 : 260-266

182. Theron, F.P. and De Kock, M.A. (1977). Clinical experience with amikacin, a new aminoglycoside antibiotic. S.A.M.J. 51 : 746-748

183. Yu, P.K. and Washington, J.A. (1977). Antimicrobial susceptibility of gentamicin resistant *Pseudomonas aeruginosa*. Mayo Clinic Proc. 52 : 802-805.

184. Reynolds, A.V., Hamilton-Miller, J.M.T., Brunfell, W. (1976). *In vitro* activity of amikacin and ten other aminoglycoside antibiotics against gentamicin resistant bacterial strains. Jnl. Infect. Dis. 134 : S291-S295

185. Saligman, S.J. (1978). Frequency of resistance to kanamycin, tobramycin, netilmicin and amikacin in gentamicin resistant and gram negative bacteria. Antimicrob. Ag. Chemo. 13 : 70-73

186. Devaud, M., Kayser, F.H. and Huber, J. (1977). Resistance of bacteria to the newer aminoglycoside antibiotics. An epidemiological and enzymatic study. Jnl. Antibiot. 30 : 655-664

187. John, J.F., Rubens, C.E. and Farrar, W.E. Jr. (1980). Characteristics of gentamicin resistance in nosocomial infections. Amer. Jnl. Med. Sc. 279 : 25-30

188. Moellering, R.C., Wennersten, C., Kunz, L.J. and Poitras, J.W. (1973). Resistance to gentamicin, tobramycin and amikacin among clinical isolates. Amer. Jnl. Med. 62 : 873-881

189. Sherris, J.C. (1970). The epidemiology of drug resistance. Proceedings of the sixth international conference on nosocomial infections. Centre for Disease Control, Atlanta, pp 50-60

190. Smith, D.H. (1967). R-factor infection of *Escherichia coli* lyophilized in 1946. Jnl. Bact. 94 : 2071-2072

191. Datta, N. (1962). Transmissible drug resistance in an epidemic strain of *Salmonella typhimurium*. Jnl. Hyg., Cambridge 60 : 301-310

192. Mare, I.J. and Coetzee, J.N. (1966). The incidence of transmissible drug resistance factors among strains of *Escherichia coli* in the Pretoria area. S.A.M.J. 40 : 980-981

193. Fried, M.A. and Vosti, K.L. (1968). The importance of underlying disease in patients with gram negative bacteraemia. Arch. Int. Med. 121 : 410-420

194. Curti, J.T. (1975). Antibiotics and gram negative bacteraemia. Jnl. Amer. Med. Assoc. 231 : 1361-1363

195. Finland, M. (1970). Changing ecology of bacterial infections as related to antibacterial therapy. Jnl. Infec. Dis. 122 : 419-431

196. Starkey, D.H. nad Gregory, E. (1971). Fluctuations in antibiotic resistances of gram negative rods in one hospital, 1961-1970, with comment on kanamycin-neomycin relationships. Canadian Med. Assoc. Jnl. 105 : 587-590

197. Whitehead, J.E.M. (1973). Bacterial resistance : Changing patterns of some common pathogens. B.M.J. 2 : 224-228

198. Finland, M., Jones, W.F., Jr. and Barnes, M.W. (1959). Occurrence of serious bacterial infections since introduction of antibacterial agents. Jnl. Amer. Med. Assoc. 170 : 2188-2197

199. Pillay, M. (1982). A study of gentamicin resistant microorganisms acquired by patients on admission to King Edward VIII Hospital. Project submitted for the Degree of Bachelor of Medical Science (Hons.), University of Durban-Westville

200. Weird, A.L., Rouchart, S., Areas, M.E. (1966). Nosocomial infection with *Klebsiella* type 25. New Eng. Jnl. Med. 275 : 17-18

201. Salzman, T.C. and Klerasm, L. (1967). Transferable drug resistance (R-factors) in *Enterobacteriaceae* : Relationship to nosocomial infections. Antimicrob. Ag. & Chemo. 1966 : 212-220

202. Adler, J.L., Shulman, J.A. and Terry, P.M. (1970). Nosocomial colonization with kanamycin resistant *Klebsiella pneumoniae*, type 2 and 11, in a premature nursery. Jnl Paediatrics 77 : 376-385.

203. Franco, J.A., Eitzman, D.V. and Baer, H. (1973). Antibiotic usage and microbial resistance in an intensive care nursery. Amer. Jnl. Dis. Child. 126 : 318-321

204. Cichon, M.J., Craig, C.P., Sargent, J. and Brauner, L. (1977). Nosocomial *Klebsiella* infections in an intensive care nursery. Southern Medical Jnl. 70 : 33-35

205. Price, D.J.E. and Sleigh, J.D. (1970). Control of infection due to *Klebsiella aerogenes* in a neurosurgical unit by withdrawal of all antibiotics. Lancet 2 : 1213-1215

206. Gardner, P. and Smith, D.H. (1969). Studies on the epidemiology of resistance (R) factors. 1. Analysis of *Klebsiella* isolates in a general hospital. 11. A prospective study of R factor transfer in the host. Annals of Inter. Med. 71 : 1-9

207. Lowbury, E.J.L., Babb, J.R. and Roe, E. (1972). Clearance from a hospital of gram negative bacilli that transfer carbenicillin resistance to *Pseudomonas aeruginosa*. Lancet 2 : 941-945

208. Bridges, K. and Lowbury, E.J.L. (1977). Drug resistance in relation to use of silver sulphadiazine cream in a burns unit. Jnl. Clin. Path. 30 : 160-164

209. Pollack, M., Charachep, P., Nieman, R.E., Jett, M.P., Reinhardt, J.A., and Hardy, P.H., Jr. (1972). Factors influencing colonization and antibiotic resistance patterns of gram negative bacteria in hospital patients. Lancet 2 : 668-671

210. Rose, H.D. and Schrier, J. (1968). The effect of hospitalization and antibiotic therapy on the gram negative fecal flora. Amer. Jnl. Med. Sc. 255 : 228-236

211. Farmer, K. (1968). The influence of hospital environment and antibiotcs on the bacterial flora of the upper respiratory tract of the newborn. New Zealand Med. Jnl. 67 : 541-544

212. Winterbauer, R.H., Turck, M. and Petersdorf, R.G. (1967). Studies on the epidemiology of *Escherichia coli* infections. V. Factors influencing acquisition of specific serologic groups. Jnl. Clin. Invest. 46 : 21-28

213. Feeley, T.W., du Monlin, G.C., Hedley-Whyte, J., Bushnell, L.S., Gilbert, J.P. and Feingold, D.S. (1975). Aerosol polymyxin and pneumonia in seriously ill patients. New Eng. Jnl. Med. 293 : 471-475

214. Swanstrom, M. and Adams, M.H. (1951). Agar layer method for production of high titre phage stocks. Proceedings of the Soc. Experi. Bio. and Med. 78 : 372-375.

215. McCabe, W.R., Kreger, B.E. and Johns, M. (1972). Type-specific and cross-reactive antibodies in gram negative bacteraemia. New Eng. Jnl. Med. 287 : 261-267

216. Roberts, A.W. and Visconti, J.A. (1972). The rational and irrational use of systemic antimicrobial drugs. Amer. Jnl. of Hospital Pharmacy 29 : 828-834

217. Kunin, C.M. (1973). Use of antibiotics. A brief exposition of the problem and some tentative solutions. Annals of Inter. Med. 79 : 555-560

218. Achong, M.R., Hauser, B.A. and Krusky (1977). Rational and irrational use of antibiotics in a Canadian teaching hospital. Canadian Med. Assoc. Jnl. 116 : 256-259

219. Scheckler, W.E. and Bennett, J.V. (1970). Antibiotic usage in seven community hospitals. Jnl. Amer. Med. Assoc. 213 : 264-267

220. Castle, M., Wilfert, C.M., Cate, T.R. and Osterhout, S. (1977). Antibiotic use at Duke University Med. Center. Jnl. Amer. Med. Assoc. 237 : 2819-2822

221. Neu, H.C. and Howrey, S.P. (1975). Testing the physician's knowledge of antibiotic use : Self-assessment and learning via videotape. New Eng. Jnl. Med. 293 : 1291-1295

222. Shooter, R.A., Faiers, M.C., Cooke, E.M., Breaden, A.L. and O'Farrell, S.M. (1971). Isolation of *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* from food in hospitals, canteens and schools. Lancet 2 : 390-392

223. Phillips, I. (1967). *Pseudomonas aeruginosa* respiratory tract infections in patient receiving mechanical ventilation. Jnl. Hyg., London 65 : 229-235

224. Casewell, M.W. and Desai, N. (1983). Sruvival of multiply-resistant *Klebsiella aerogenes* and other gram negative bacilli on finger-tips. Jnl. Hosp. Infect. 4 : 350-360

225. Salzman, T.C., Clark, J.J. and Klemm, L. (1968). Hand contamination of personnel as a mechanism of cross infection in nosocomial infections with antibiotic resistant *Escherichia coli* and *Klebsiella aerobacter*. Antimicrobial Ag. & Chemo. 1967 : 97-100

226. Knittle, M.A., Eitzman, D.V. and Baer, H. (1975). Role of hand contamination of personnel in the epidemiology of gram negative nosocomial infections. Jnl. Paediatrics 86 : 433-437

227. Adams, B.G. and Marrie, T.J. (1982). Hand carriage of aerobic gram negative rods by health care personnel. Jnl. Hyg. 89 : 23-31

228. Larson, E.L. (1981). Persistent carriage of gram negative bacteria on hands. Amer. Jnl. Infect. Control 9 : 112-119

229. McBride, M.E., Duncan, W.C. and Knox, J.M. (1975). Physiological and environmental control of gram negative bacteria on skin. Brt. Jnl. Dermatology 93 : 191-199

230. Ayliffe, G.A.J., Babb, J.R. and Quoraishi, A.H. (1978). A test for "hygienic" hand disinfection. Jnl. Clin. Path. 31 : 923-928

231. Cooke, E.M., Edmondson, A.S. and Starkey, W. (1981). The ability of strains of *Klebsiella aerogenes* to survive on the hands. Jnl Med. Micro. 14 : 443-450

232. Bruun, J.N. and Solberg, C.O. (1973). Hand carriage of gram negative bacilli and *Staphylococcus aureus*. B.M.J. 2 : 580-582

233. Casewell, M.W. and Phillips, I. (1978). Epidemiological patterns of *Klebsiella* colonization and infection in an intensive care ward. Jnl. Hyg., Cambridge 80 : 295-299

234. Sprunt, K., Redman, W. and Leidy, G. (1973). Antibacterial effectiveness of routine hand washing. Paediatrics 52 : 264-271

235. Mortimer, E.A., Wolinsky, E., Gonzaga, A.J. and Rammelkamp, C.H. (1966). Role of air-born transmission in staphylococcal infections. B.M.J. 1 :319-322

236. Maples, R.R.and Towers, A.G. (1979). A laboratory model for the investigation of contact transfer of micro-organisms. Jnl. Hyg. 82 : 237-248

237. Burke, J.P., Ingall, D. Klein, J.O., Gezon, H.M. and Finland, M. (1971). *Proteus mirabilis* infections in a hospital nursery traced to a human carrier. New Eng. Jnl. Med. 284 : 115-121

238. Morehead, C.D. and Houck, P.W. (1972). Epidemiology of *Pseudomonas* infections in a paediatric intensive care unit. Amer. Jnl. Dis. Child. 124 : 564-570

239. Steere, A.C. and Mallison, G.F. (1975). Handwashing practices for the prevention of nosocomial infections. Annals of Inter. Med. 83 : 683-690

240. Ransjo, U. (1979). Attempts to control clothes-borne infections in a burns unit. 2. Clothing routines in clinical use and the epidemiology of cross-colonization. Jnl. Hyg., Cambridge 82 : 369-384

241. Hambraeus, A. and Ransjo, U. (1977). Attempts to control clothes-borne infection in a burns unit, 1. Experimental investigations of some clothes for barrier nursing. Jnl. Hyg. Cambridge 79 : 193-202

242. Nystrom, B. (1981). The contamination of gowns in an intensive care unit. Jnl. Hosp. Infect. 2 : 167-170

243. Babb, J.R., Davies, J.G.and Ayliffe, G.A.J. (1983). Contamination of protective clothing and nurses' uniforms in an isolation ward. Jnl. Hosp. Infect. 4 : 149-157

244. Hambraeus, A. (1973). Transfer of *Staphylococcus aureus* via nurses' uniforms. Jnl.Hyg. Cambridge 71 : 799-814

245. Whitby, J.L. and Rampling, A. (1972). *Pseudomonas aeruginosa* contamination in domestic and hospital environment. Lancet 1 : 15-17

246. Rubbo, D.S., Gardner, J.F.and Franklin, J.C. (1966). Sources of *Pseudomonas aeruginosa* infection in premature infants. Jnl. of Hyg., Cambridge 64 : 121-128

247. Bassett, D.J., Thompson, S.A.S. and Page, B. (1965). Neonatal infections with *Pseudomonas aeruginosa* associated with contaminated resuscitation equipment. Lancet 1 : 781-784

248. Drewett, S.E., Payne, D.J.H., Tuke, W. and Verdon, P.E. (1972). Eradication of *Pseudomonas aeruginosa* infection from a special care nursery. Lancet 1 : 946-948

249. Cooke, E.M., Shooter, R.A., O'Farrell, S.M. and Martin, D.R. (1970) Faecal carriage of *Pseudomonas aeruginosa* by newborn babies. Lancet 2 : 1045-1046

250. Brown, D.F. and Baublis, J. (1977). Reservoirs of *Pseudomonas aeruginosa* in an intensive care unit for newborn infants : mechanisms of control Jnl. Paed. 90 : 453-457

251. Ayliffe, G.A.J., Babb, J.R., Collins, B.J. Lowbury, E.J. and Newsom, S.W.B. (1974). *Pseudomonas aeruginosa* in hospital sinks. Lancet 2 : 578-581

252. Al-Dukaili, A.H. and Harris, D.M. (1975). *Pseudomonas aeruginosa* in hospital : a comparison between 'infective' and 'environmental' strains. Jnl. Hyg. Cambridge 75 : 195-201

253. Chadwick, P. (1976). The epidemiological significance of *Pseudomonas aeruginosa* in hospital sinks. Canadian Jnl of Public Health 67 : 323-328

254. Editorial (1976). Brit. Jnl. Anaest. 41 : 1

255. Joseph, J.M . (1952). Disease transmission by inefficiently sanitized anesthetizing apparatus. Jnl Amer. Med. Assoc. 147 : 1196-1198

256. Rogers, K.B. (1960). *Pseudomonas* infections in a children's hospital. Jnl. Applied Bact. 23 : 533-537

257. Magath, T.B. (1938). Method for preventing cross infection with gas machines. Anaesthesia and Analgesia Current Researches 17 : 215-217

258. Hoffmann, M.A. and Finberg, L. (1955). *Pseudomonas* infections in infants associated with high-humidity environments. Jnl Paed. 46 : 626-630

259. Macpherson, C.R. (1958). Oxygen therapy - an unsuspected source of hospital infections? Jnl. Amer. Med. Assoc. 167 : 1083-1086

260. Phillips, I. and Spencer, G. (1965). *Pseudomonas aeruginosa* cross-infection due to contaminated respiratory apparatus. Lancet 2 : 1325-1327

261. Reinarz, J.A., Pierce, A.K., Mays, B.B. and Sandord, J.P. (1965). The potential role of inhalation therapy equipment in nosocomial pulmonary infection. Jnl. Clin. Investigation 44 : 831-839

262. Mertz, J.J., Scharer, L. and McClement, J.H. (1967). A hospital outbreak of *Klebsiella pneumonia* from inhalation therapy with contaminated aerosol solutions. Amer. Reviw of Respiratory Dis. 95 : 454-460.

263. Goodison, R.R. (1980). *Pseudomonas* cross infection due to contaminated humidifier water. B.M.J. 281 : 1281

264. Rapkin, R.H. (1976). *Pseudomonas cepacia* in an intensive care nursery. Paediatrics 56 : 239-243

265. Redding, P.J. and McWalter, P.W. (1980). *Pseudomonas fluorescens* corss infection due to contaminated humidifier water. B.M.J. 281 : 275

266. Sutter, V.L., Hu-st, V., Grossman, M. and Calonje, R. (1966). Source and significance of *Pseudomonas aeruginosa* in sputum. Patients requiring tracheal suction. Jnl. Amer. Med. Assoc. 197 : 854-858

267. Moffet, H.L. and Williams, T. (1967). Survival and dissemination of bacteria in nebulizers and incubators. Amer. Jnl. Dis. Child. 114 : 13-20

268. Edmondson, E.B., Reinarz, J.A., Pierce, A.K. and Sanford, J.P. (1966). Nebulization equipment : a potential source of infection in gram negative pneumonias. Amer. Jnl. Dis. Child. 111 : 357-360

269. Moffet, H.L. and Allan, D. (1967). Colonization of infants exposed to bacterially contaminated mists. Amer. Jnl. Dis. Child. 114 : 21-25

270. Grieble, H.G. Colton, F.R., Bird,T.J. Toigo,A. and Griffith, L.G. (1970). Fine-particle humidifiers. Sources of *Pseudomonas aeruginosa* infections in a respiratory disease unit. New Eng. Jnl. Med. 282 : 531-535

271. Babington,P.C.B., Baker, A.B. and Johnston, H.H. (1971). Retrograde spread of organisms from ventilator to patient via the expiratory limb. Lancet 1 : 61-62

272. Kelsen, S.G., McGuckin, M., Kelsen, D.P. and Cherniack, N.S. (1977). Airborne contamination of fine-particle nebulizers. Jnl. Amer. Med. Assoc. 237 : 2311-2314

273. Sykes, M.K. (1972). Sterilization of ventilators. International Anaesthesiology Clinics 10 : 131-146

274. Sanders, C.V., Luby, J.P., Johanson, W.G., Barnett, J.A. and Sanford, J.P. (1970). *Serratia marcescens* infections from inhalation therapy medication : nosocomial outbreak. Annals of Intern. Med. 73 : 15-21

275. Johanson, W.G., Pierce, A.K., Sanford, J.P. and Thomas, G.D. (1972). Nosocomial respiratory infections with gram negative bacilli. The significance of colonization of the respiratory tract. Annals of Inter. Med. 77 : 701-706

276. Northey, D., Adess, M.L., Hartsuck, J.M. and Rhoades, E.R. (1974). Microbial surveillance in a surgical intensive care unit. Surgery, Gynaecology and Obstetrics 139 : 321-325

277. Pierce, A.K. and Sanford, J.P. (1974). Aerobic gram negative bacillary pneumonias. Amer. Review of Respiratory Dis. 110 : 647-658

278. Stevens, R.M., Teres, D., Skillman, J.J. and Feingold, D.S. (1974). Pneumonia in an intensive care unit. Arch. of Inter. Med. 134 : 106-111

279. Johanson, W.G., Pierce, A.K. and Sanford, J.P. (1969). Changing pharyngeal bacterial flora of hospitalized patients. Emergence of gram negative bacilli. New Eng. Jnl. Med. 281 : 1137-1140

280. Philip, J.R. and Spencer, R.C. (1974). Secondary respiratory infection in hospital patients : effect of antimicrobial agents and environment. B.M.J. 2 : 359-362

281. Atherton, S.T.and White, D.J. (1978). Stomach as a source of bacteria colonising respiratory tract during artificial ventilation. Lancet 2 : 968-969

282. Johanson, W.G., Woods, D.E. and Chaudhuri, T. (1979). Association of respiratory tract colonization with adherence of gram negative bacilli to epithelial cells. Jnl. Infect. Dis. 139 : 667-673

283. Laforce, F.M. (1981). Hospital acquired gram negative rod pneumonias : an overview. Amer. Jnl. Med. 70 : 664-669

284. Jellard, C.H. and Churcher, G.M. (1967). An outbreak of *Pseudomonas aeruginosa* (pyocyanea) infection in a premature baby unit, with observations on the intestinal carriage of *Pseudomonas aeruginosa* in the newborn. Jnl. Hyg. Cambridge 65 : 219-228

285. Fierer, J., Taylor, P.M. and Gezon, H.M. (1967). *Pseudomonas aeruginosa* epidemic traced to delivery-room resuscitators. New Eng. Jnl. Med. 276 : 991-996

286. Dexter, F. (1971). *Pseudomonas aeruginosa* in a regional burns centre. Jnl. Hyg. Cambridge 69 : 179.

287. Damaso, D. and Moreno-Lopez, M. (1973). Study on *in vitro* activity of the antibiotics tobramycin and gentamicin against *Pseudomonas aeruginosa* in clinical strains. J. Antibiot. 26 : 233-237

288. Duncan, I.B.R. (1974). Susceptibility of 1,500 isolates of *Pseudomonas aeruginosa* to gentamicin, carbenicillin, colistin and polymyxin B. Antimicrobial Ag. Chemo. 5 : 9-15

289. Fullbrook, P.D., Elson, S.W. and Slocombe, B. (1970). R-factor mediated B-lactamase in *Pseudomonas aeruginosa* . Nature (London) 226 : 1054-1056

290. Habs, I. (1957). Untersuchungen uber die O. antigene von *Pseudomonas aeruginosa*. Z. Hyg. Infektionskr. 144 : 218-228

291. Veron, M. (1961). Sur I'agglutination de *Pseudomonas aeruginosa* : subdivision des groups antigeniques O:2 et O:5. Ann. Inst. Pasteur, 101 : 456-460

292. Bergan, T. (1975). Epidemiological typing of *Pseudomonas aeruginosa*. In : Resistance of *Pseudomonas aeruginosa*. J. Wiley & Sons, London, pp. 189-235