# TRANSFERABLE DRUG RESISTANCE IN THE GRAM-NEGATIVE FLORA AND THE IMPLICATION IT HAS ON THE TREATMENT OF URINARY TRACT INFECTIONS AT MUHIMBILI MEDICAL CENTRE

BY

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TO SUSAN, MWAYAONA, MY MOTHER SALOME

AND

FATHER ALEXANDER

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## ABBREVIATIONS

| | |
|---|---|
| Amp | ampicillin |
| Car | carbenicillin |
| C.P.L. | Central Pathology Laboratory |
| Chl | Chloramphenicol |
| Cot | cotrimoxazole |
| D.M.J. | Dar es Salaam Medical Journal |
| D.N.A. | Deoxyribonucleic acid |
| D.S.T. | Diagnostic Sensitivity Agar |
| Gen | gentamycin |
| Kan | kanamycin |
| $Lac^+$ | Lactose positive phenotype |
| $Lac^-$ | Lactose negative phenotype |
| L.L.F. | Late lactose fermenter |
| M.C.A. | MacConkey's Agar |
| M.R.V.P. | Methyl-red and voges-proskauer |
| N.A. | Nutrient Agar |
| Nal | nalidixic acid |
| $Nal^R$ | Chromosomally coded nalidixic acid resistance |
| N.B. | Nutrient broth |
| Nit | nitrofurantoin |
| N.D. | Not done |
| O.N. | Overnight |
| PABA | Para-aminobenzoic acid |
| $R^-$ | Recipient phenotype without R-plasmid |
| RNA | Ribonucleic acid |
| S.S. | Salmonella/Shigella |
| S.T.T. | Sensitive to all drugs tested |
| Str. | Stereptomycin |
| $Str^R$ | Chromosomally coded streptomycin resistance |
| Sud | Sulphadiazine |
| Sul | Sulphonamide |
| Tet | tetracycline |
| T.F. | Transfer factor |
| Tra | Transfer operon |
| Tri | trimethoprim |
| T.S.I. | Tripple Sugar Iron agar |
| U.K. | United Kingdom |
| U.T.I. | Urinary tract infection |
| W.H.O. | World Health Organization |

## SUMMARY

Isolates resistant to various antibiotics were obtained from normal health human subjects in Birmingham (U.K.) and Dar es Salaam (Tanzania). The level of resistance observed was found to be quite high for both Dar es Salaam and Birmingham. Twenty four (24) percent or more of all isolates were resistant to 3 or more antibiotics regardless of whether the isolates were from Dar es Salaam or Birmingham.

An attempt was made to transfer these resistances (assumed to be extrachromosomal) to known recipients E.coli K12S and E.coli K12N, Isolates from patients with urinary tract infections from Dar es Salaam were also used in the transfer experiments as donors. The procudure was repeated using 'unknown donors' and 'unknown recipients'

E.coli K12 W3110 containing R-plasmid RP1 was used to monitor transfer efficiency. Transfer experiments were performed at 28°C or 37°C and in ashaking or stationary waterbath. This was in order to achieve the most efficient transfer and control for potentially temperature sensitive transfer mechanisms in donor strains.

There was a marked difference in the transfer ability of R-factors between isolates from Birmingham and those from Dar es Salaam; the latter being higher. Transfer between unknown donors and unknown recipients was observed. However, this was extremely low.

Transfer of drug resistance in Dar es Salaam isolates was used to delineate the role of R-factors in the increasing bacterial resistance to drugs used in the treatment of urinary tract invections at the Muhimbili Medical Centre.

# INTRODUCTION

# I. INTRODUCTION

The increasing frequency of acquired resistance to antibiotics is a World-wide health problem which demands attention. However, the rapidity with which new resistant strains are appearing, and the fact that existing resistant strains are becoming more prevalent, highlight the need for more information about the current situation and action to control it.

The discovery of antibiotics was perhaps one of the most significant development in the history of public health. Antibiotics saved millions of lives and shortened the duration of illness for hundreds of millions. We owe chemotherapy the debt of reducing the high mortality rate of many bacterial infections. However, the dramatic nature of their effects encouraged an explosive increase in their use both for human and in verterinary medicine. All this contributed to the growing problem of bacterial resistance.

The first clinically serious consequence of antibiotic resistance was the widespread dissemination in hospital, in the 1950's, of strains of Staphylococcus aureus that were resistant to penicillin. These strains had developed the ability to form an antibiotic destroying enzyme (Beta - lactamase) and they subsquently acquired resistance to several other chemically unrelated antibiotics. From 1950s onwards, these so called "multiple-antibiotic" resistant staphylococci became endemically established in many hospitals throughout the world.

Recently the situation has worsened. Surveillance data presented to the WHO (Willard, N. 1984) indicate that serious consequences of antibiotic resistance are no longer confined to hospitals but are increasing in the general population.

The prevalence is even greater in developing countries than in industrialized ones. Resistance to such easily available antibiotics as ampicillin, tetracycline, chloramphenicol and sulphonamides has made its appearance. Patients in developing countries are now in a situation where only the low cost antibiotics are available to them, yet these are becoming progressively less effective. Antibiotic resistance limits the effectiveness of antibiotics against bacteria. These bacteria may be resistant to the antibiotic from the start, or they may acquire resistance from another organism in the patient during treatment.

Resistant bacteria may actually grow faster in the presence of antibiotics (Willard, N. 1984). This can have important clinical consequences, especially in hospital patients, many of whom have an increased susceptibility to infection by organisms that seldom invade healthy persons. These organisms are frequently responsible for respiratory or septicemic complications that may be a greater hazard than the infection for which the antibiotic treatment was originally given.

The use of wide-spectrum antibiotics, particularly when not essential, has produced resistance in a wide variety of bacteria, thus seriously limiting the possibilities of controlling infection by any of the existing antibiotics (Willard, N. 1984).

The fact that drug resistant bacteria have considerably increased in number in recent years among various species of bacteria in parallel with the extensive use of chemo-therapeutic agents is very well known. The frequencies of drug resistant bacteria are particularly high among Gram-negative enteric bacteria. (Allison et al 1969, Dalton and Allison 1967).

Following the introduction of a number of chemotherapeutic agents and antibiotics, many observations on bacterial drug resistance were reported as shown by reports of resistance to sulphonamides by Maclean et al (1939) to penicillin by Abraham et al (1941) and to streptomycin by Murray et al (1964). Soon after the discovery of Sulphanilamide it was found that the derivatives of sulphanilamide were effective against bacillary dysentery as well as the infectious diseases caused by Gram-positive bacteria, and a large amount of sulphanilamide was used in Japan since 1945. However, its effectiveness had a duration of only about five years and sulphanilamide resistant shigella strains appeared rapidly and reached a maximum of about 80-90 percent of Shigella isolates being resistant. Fortunately the production of antibiotics such as streptomycin, tetracycline and chloramphanicol, started at the same time, and were quite effective against bacillary dysentery. Streptomycin production commenced in 1950 and by 1953 its production increased rapidly to meet the demand; tetracycline was imported from the United States and Germany in 1950. In 1954 the production of chloramphenicol increased rapidly in spite of a large amount of sulphanilamide and tetracycline production. With the increase of antibiotic production, antibiotic - resistant Shigella strains appeared and have gradually increased. The first isolation of a multiple resistance Shigella strain occured in 1952. This multiple resistance involved tetracycline, sulphanilamide and Streptomycin. By 1955 a quadruple resistant Shigella strain resistant to tetracycline, streptomycin, sulphanilamide and chloramphenicol had been isolated from a woman afflicted with bacillary dysentery (Mitsuhashi, S. 1971). This phenomenon has now been demonstrated in all the enterobacteria, including non-pathogenic Escherichia coli.

It has also been found in many Shigella strains outside Japan, including the Sh. dysenteriae type I which caused the immense outbreak in central America and which is believed to have caused over a hundred thousand deaths from 1968 onwards; in the large Mexican typhoid outbreak which started in 1972 and produced many thousands of cases, with salmonellosis in animals and man in many parts of the World; with enteropathogenic E.coli in outbreaks of enteritis in young animals and human infants; and with exacerbations of infections with opportunist pathogens such as Klebsiella, Pseudomonas, Proteus and Serratia in many specialized paedriatric and geriatric intensive care units (W.H.O. 1976).

## 1.1. PRESSURES FAVOURING EXCESSIVE ANTIBIOTIC USE:

When antibiotics are available on the open market, the attitude of patient and family are decisive. The desire to do the best for the patient in a situation of fear and anxiety, coupled with public ignorance about the efficacy of antibiotics in particular diseases, leads to unnecessary and even damaging, treatment. In other cases the dosage is inappropriate. Either insufficient or not taken long enough. Poor choice of antibiotics often results from the multiplicity of names under which they are marketed, the promotion in developing countries of antibiotics that are obsolete or inappropriate and misleading advertizing.

Unless physicians have a good knowledge of management of microbial infections, they may be tempted to give unnecessary treatment, in an effort to do the best for the patient.

Moreover, recent surveys in North America and Britain indicate that about one-quarter of all patients receive one or more courses of antibiotics while in hospital. In a Canadian Survey in 1976 (Willard, N. 1984) only 41% of all courses of antibiotics that were prescribed could be considered as "rational" and 22% were "questionable".

In hospitals, the most frequent form of therapeutic misuse is to give unnecessary courses of antibiotics. This is true in general practice as well; in one Survey in USA, nearly 60% of physicians used antibiotics to treat the common cold, which is a virus infection no antibiotic can combat (Willard N. 1984).

## 1.2 RESISTANCE TO ANTIMICROBIAL DRUGS:

There are many different mechanisms by which micro-organisms exibit resistance to drugs. Examples include:-

1. Production of enzymes that destroy the active drug. Staphylococci resistant to penicillin G produce a beta-lactamase that destroys the drug. Other beta lactamases are produced by gram-negative rods. Gram-negative bacteria resistant to amino-glycosides (by virtue of a plasmid) produce adenylating, phosphorylating or acetylating enzymes that destroy the drug. Gram-negative bacteria may be resistant to chloramphenicol if they produce a chloramphenicol acetyltransferase.
2. Microorganisms change their permeability to the drug. For example, tetracyclines accumulate in susceptible bacteria but not in resistant bacteria. Resistance to polymyxins is also associated with a change in permeability barrier to aminoglycosides. This can be partly overcome by the simultaneous presence of a cell wall active drug, eg. a penicillin. Resistance to amikacin and to some other aminoglycosides may depend on a lack of permeability to the drug, apparently a mutational event.
3. Microorganisms develop an altered structural target for the drug. For example, chromosomal resistance to aminoglycosides is associated with the loss or alteration of a specific protein on the 30s subunit of the bacterial chromosome that serves as a binding site in susceptible organisms. Erythromycin resistant organisms have an altered receptor on the 50s subunit of ribosomes, resulting from methylation of a 23s ribosomal RNA.

4. Microorganisms develop an altered metabolic pathway that by-passes the reaction inhibited by the drug. Some sulphonamide resistant bacteria do not require extracellular Para-Amino-Benzoic Acid (PABA) but, like mammalian cells, can utilize preformed folic acid.

5. Microorganisms develop an altered enzyme that can still perform its metabolic function but is much less affected by the drug than the enzyme in the susceptible organism. In sulphonamide susceptible bacteria, the tetrahydropteroic acid synthetase has a much higher affinity for sulphonamide than for PABA. In sulphonamide resistant mutants the reverse is true.

In fact there may be two types of drug resistance in bacteria, genetic and non-genetic in origin. As is well known, active replication of bacteria is usually required for most antibacterial drug actions. As a result, organisms that are metabolically inactive may be phenotypically resistant to drugs. However, their offspring are fully susceptible. Examples include mycobacteria, which often survive in tissues for many years after infection yet are restrained by hosts defenses and do not multiply. Such organisms are resistant to treatment and cannot be eradicated by drugs. Yet if they start to multiply as occurs when following corticosteroid treatment of the patient, they are fully susceptible to the same drug.

Microorganisms may also lose the specific target structure for a drug for several generations and thus be resistant. For example, penicillin-susceptible organisms may change to L-forms during penicillin administration. Lacking most cellwall, they are then resistant to cell-wall inhibitor drugs and may remain so for several generations.

When the same organisms revert to their bacterial parent forms by resuming Cellwall production, they are again fully penicillin-susceptible. (Jawetz E. et al 1978)

Genetic resistance may either be constitutive or inducible. Drug-resistance in the former type is unstable and high resistance is demonstrated only when the strain is pretreated with sub-inhibitory concentrations of the drug. The resistance of the induced cells is lost when grown in the absence of inducers. In other words, the antibacterial agent is the active inducer and the properties of inducible resistance are inheritable. Both types of resistance are based on mutation and can be accounted for by present concepts in genetics. A resistant variant appears spontaneously during the course of bacterial multiplication, without regard to the presence or absence of the drug. The drug acts merely as a selective agent which inhibits the cells that have failed to develop the mutation but allows the growth of a mutant that has developed the appropriate mutation.

The genetic concept can easily be applied to the instance of one-step mutation; For example, a single-step pattern of high resistance to streptomycin was demonstrated by the fluctuation analysis.

On this basis one would expect that bacterial resistance in vivo to streptomycin would be a common event and indeed it is established that the rapid emergence of resistant strains to this drug forms the main obstacle to its successiful use clinically. Other studies in vitro showed that a single mutational event often resulted in only a slight increase in drug resistance and that a number of sequential mutations were usually required before a sensitive organism developed a high level of resistance. This multistep pattern of accumulating resistance by successive increments was characteristic for penicillin, tetracycline and chloramphenicol.

Definitive evidence of the problem of bacterial resistance was furnished by the fluctuation analysis and later by the replica-plating methods; The results of both experiments indicated that drug resistant-mutants appear spontaneously before exposure to the drug. The drug only acts as a selective agent for drug-resistant mutants.

Many drug resistant strains of bacteria reported in earlier papers were obtained *in vitro* under experimental conditions. Bacteria may be rendered drug-resistant by single, repeated or continuous exposure to antibacterial agents *in vitro* or *in vivo* using experimental animals, even though the mutation rates differ for each strain. Moreover, it has also been noticed that in the genetic and biochemical mechanisms of bacterial drug-resistance there are many differences between resistant strains obtained in the laboratory and those isolated from clinical sources. For instance Staphylococcal strains which are rendered resistant to penicillin in *in vitro* experiments, do not inactivate the drug by beta-lactamase, while many wild strains isolated from clinical sources have high beta-lactamase activity, the main factor responsible for resistance to that drug (Geronimus and Cohen 1957). By repeated exposure of Staphylococci to chloramphenicol, the strains become resistant to this drug but the mechanism of chloramphenicol resistance cannot be accounted for by inactivation of the drug (Suzuki *et al* 1966; Kono *et al* 1968). Similar results were obtained in gram-negative bacteria and for Pseudomonas by Foster *et al* (1981); Mitsuhashi *et al* (1975) and Foster and Dorman (1982). Phillips *et al* (1978) also investigated gentamycin-resistant strains of Pseudomonas for the presence of inactivating enzymes, of 44 isolates of *P.auroginosa* resistant to gentamycin 10 produced inactivating enzymes, 4 strains produced an enzyme that did not account for their resistance to gentamycin.

The remainder did not produce inactivating enzymes but showed increased resistance to all aminoglycosides including amikacin and tobramycin. A 5.9 megadalton plasmid which does not code for beta-lactamase in Haemophilus ducreyi was also reported by Thomson and Bilgeri (1982). In clinical cases however, Staphylococcal strains which are resistant to chloramphenicol inactivate it by acetylation using intrinsic acetyl Co A. (Suzuki et al 1966); (Kono et al 1968).

## 1.3. PLASMIDS: DEFINITION

Another important factor responsible for the wide spread of multiple resistance is gene transfer from cell to cell among various species of bacteria. R-factors have been cited as the cause of the increase in multiple drug resistance by various investigators. The R-factor (Plasmid) is similar to the sex factor F(F-factor); like the F-factor it consists of deoxyribonucleic acid. The genetics of R-factors have been studied extensively. It appears to be composed of one or more genes promoting replication and conjugation known as resistant transfer factor (RTF) together with the genes causing resistance to one or more drugs (R-determinants).

By using bacterial strains with various chromosomal markers, Watanabe and Fukasawa (1961) found that the transfer of resistance usually occurs without the transfer of the chromosomal markers. This is good evidence that the transferable drug resistance factors are extrachromosomal or cytoplasmic elements. The kinetics of transfer of drug resistance in populations of drug sensitive bacteria also suggest that the responsible factors replicate faster than do bacterial chromosomes, since the number of recipient cells becoming drug resistant increase sharply.

Plasmids can be defined as extrachromosomal DNA elements of bacteria that constitute a reasonably stable, but dispensable gene pool. Under most circumstances they are not essential for the successiful growth and metabolism of their host bacterium. Often, however, these genetic elements carry genes for some supplementary activity that allows their host to better survive in adverse environments or permit their hosts to compete more successfully with other microorganisms of the same or different species. The best known plasmids from the stand - point of human medicine are those that specify antibiotic resistances (Starr et al 1980).

They may code for single or multiple resistances. Their resistance is directed against the antibacterial drugs used in human and veterinary medicine and in some countries also for growth promotion in livestock, pigs, poultry and cattle (W.H.O. 1976). It has become increasingly apparent, however, that plasmids also specify properties that directly contribute to the pathogenicity of a variety of microorganisms Starr et al (1980).

Classification of R-factors:

R - factors can be broadly divided into two classes.

Class I R-factors:

In Class I R-factors, a genetic determinant for drug resistance (R-determinant) is integrated into the transfer factor (TF) which is responsible for its mobility, to form a single covalently - bonded plasmid which is transferred as an intact linkage group from donor to recipient bacteria. These recipients in turn become donors and can transfer the complete R-factor to new recipients. The transferability of the complex is usually stably inherited.

Class I R-factors may carry single or multiple-resistances; though some resistances are easily lost probably by deletion, others are stable. Loss of the transfer factor is usually associated with loss of the entire R factor.

In class II R-factors, the resistance determinant and the transfer factor are separate plasmids, independent of each other in the host cell. They are apparently associated with each other only in transfer, although the nature of the association is as yet undetermined.

In class II transfer systems, a number of determinants may be independently associated with a single transfer factor in the host cell, and in the absence of multiple selection they are transferred independently to recipient strains.

The resistances of the donor will thus segregate during transfer. Because of their independence, the R-determinants and transfer factors of class II Systems can be lost individually.

R-determinants in class II systems are usually transferred at a much lower frequency ($10^{-2}$ or less) than that of the transfer factor, which may infect up to 100% of recipient cells in an overnight culture of mating mixtures (crosses).

The structure of class II systems makes it easy to separate the components in crosses. If, for example, the donor strain carries an Str-Sul determinant and a suitable TF, recipient lines can be isolated that carry: Str-Sul only, Str-Sul and TF, and the TF only. WHO (1976).

Antibiotic resistance in bacteria may be mediated in a variety of ways, but whatever its mechanism, or its genetic control, it is usually found that in the absence of antibiotic usage, antibiotic-resistant organisms form only a minority population. Mare' (1968) studied bushmen and wild animals in Rhodesia. The bushmen had not been in touch with other human beings for ten years. Ten percent of the specimens examined from both sources contained drug resistant gram-negative bacteria, many with multiple resistance. However, this antibiotic resistance was not transferable and whether it was mobilizable in the presence of a transfer factor was not investigated. In the Solomon Islands Gardener et al (1969) studied gram-negative bacilli isolated from faeces and from soil. From nineteen faecal specimens one R factor carying E.coli was isolated and from 21 soil specimens, one R factor carying alcaligenes-like species. Other workers studying similar isolated communities, have obtained the same type of results.

North Borneo has also been investigated (Davis and Anandan (1970). From four of 128 people from whom faeces were obtained, six strains of E.coli which carried R-factors were isolated.

When antibiotics are used, selective pressure in favour of antibiotic resistant organisms is exerted and the proportion of these in the bacterial population rises. For example following the introduction of penicillin G, more than seventy percent of Staphyloccoccus aureus strains causing serious hospital infections were resistant within three years (Munch-Petersen and Boundy, 1962). The importance of selection pressure is reviewed by Richmond (1972).

There is no evidence that chemotherapeutic agents induce the development of R-factors, but even in communities in which antibiotics are not used, there may be some selective pressure by the products of the streptomyces present in the soil.

Following the discovery of F, a large number of bacterial host properties have been found to be determined by plasmid-carried genes. Most plasmids have been classified on the basis of the host properties which initially led to their detection. Thus there are the R-factors (for resistance) and Col factors (for 'Colicinogeny') of gram-negative bacteria, the penicillinase plasmids of Staphyloccoccus aureus, and the cryptic plasmids.

Those plasmids which confer on their host the ability to transfer chromosomal markers, but no other readily detectable properties have been called F factors; this group includes the original F factor discovered in E.coli $K_{12}$, which has been designated as F 1.

Many plasmids however, including some R-factors and Col factors can mobilize the chromosome for transfer, and some authors have used the term sex factor to describe any plasmid with this potential. The term sex factor has also been used in two other ways:

(a) as a generic name for all plasmids which determine host conjugation and thus their own transfer, regardless of whether or not chromosomal markers are also transferred and

(b) to describe that set of genes on the plasmid whose products mediate the conjugational process. (Stanier et al 1978).

## 1.4 MECHANISMS OF GENE TRANSFER

The methods of gene transfer between organisms have been described in a number of symposia, Wolstenholme and O'connor (1969); Dulonoy and Laskin (1971). Zygote formation in eucaryotic cells results from the fusion of the gametes of sexually compatible members of the same species. Zygotes are formed in bacteria by more primitive means of gene transfer. Three general machanisms are known whereby the genetic information of one bacterial cell can be introduced into a recipient cell.

Transformation:

This is the most primitive mode of transfer (Stanier et al 1978) in which naked DNA derived from one cell is taken up by another cell. The method had been shown to occur in the genera Bacillus, Neisseria, Haemophilus and Pneumococcus. It is probably not important in antibiotic resistance.

## Transduction

A temperate bacteriophage transfers genetic material the donor to recipient. It occurs in a large number of gram-negative organisms including *E.coli* and also occurs in Staphylococci and in the Bacillus species.

Transduction may involve the transfer to the donor chromosome only of those segments immediately adjacent to the attachment of the prophage (Restricted transduction) or other segments may stand an equal chance of being transferred (generalized transduction). Once transduced the exogenote may be integrated into the host chromosome and replicate with the host. However, if it is not integrated it may persist without replication; this is abortive transduction. In Staphylococci, transduction may be important in the spread of resistance to streptomycin, tetracycline, chloramphenicol, and erythromycin. Most of the transduced elements are extrachromosomal but transduction of chromosomal elements has also been shown to occur. Transduction occurs *in vivo* as well as *in vitro*.

The importance of transduction in the spread of antibiotic resistance in *E.coli* is not known.

## Conjugation

In conjugation, there is evidence that the mechanism is important. It is also interesting because genetic transfer can occur between different species of organisms and because transfer of resistance to several antibiotics can occur at the same time. Moreover, conjugation is probably most analogous to fusion of gametes, where genetic transfer is preceded by cell to cell contact. The latter process does not involve cell fusion. Incomplete, rather than full, diploids result from gene transfer in bacteria.

These partial diploids are known as merozygotes and consist of the entire genome (endogenote) derived from the recipient cell and the incomplete genome (exogenote) derived from the donor cell. Regardless of the mode of gene transfer, merozygotes support genetic recombination in which portions of the DNA of the exogenote effectively replace segments of the DNA endogenote. (Stanier et al 1978).

Because of the importance in antibiotic resistance, conjugation will be dealt with in a much more detailed approach than transduction and transformation. Furthermore, the fact that the experimental work of this thesis is based mainly on bacterial conjugation makes it even more appropriate to have a detailed account of recent developments in the field.

## 1.5. BACTERIAL CONJUGATION:

Conjugation is the highly specific process whereby DNA is transferred from donor to recipient bacteria by a mechanism involving cell to cell contact. This process is usually encoded by conjugative plasmids, which have been isolated from a diverse range of gram-negative bacteria and include members of more than twenty incompatibility groups. All these plasmid encode conjugative pili necessary for establishing cell to cell contact, and their molecular mechanisms of conjugation may be similar. However, it is likely that many genetically distinct conjugation systems exist, since plasmids from one incompatibility group/family do not complement transfer deficient mutants of another. Also plasmids belong to different incompatibility groups generally show little DNA homology (Willets and Wilkins 1984).

### Transfer of DNA:

The fate of two component DNA strands of a plasmid during conjugation was first analyzed with F, and this plasmid remains the best documented in this regard. The finding that minicell recipients accumulated predominantly single stranded DNA during F-mediated conjugation suggested that only one strand of the plasmid is transferred. One strand of plasmid DNA is broken at the replication origin and the dublex unwinds, the broken strand entering the recipient cell beginning with its 5' terminus (Stanier et al 1978) (Fig. 1). Subsequently it was found that this strand of F is the denser of the two in polyuridylate - polyguanylate (Poly U, G)-Cs-Cl gradients and that the lighter is retained in the donor cell. By selectively labelling DNA in either the donor or the recipient cell, it was further demonstrated that DNA is synthesized on the transferred F strand in the recipient and that the strand retained in the donor serves as template for synthesis of DNA to replace that transferred.

Although the transfer of one strand of plasmid DNA duplex is usually accompanied by replication, such replication is not essential for the transfer process (Stanier et al 1978).

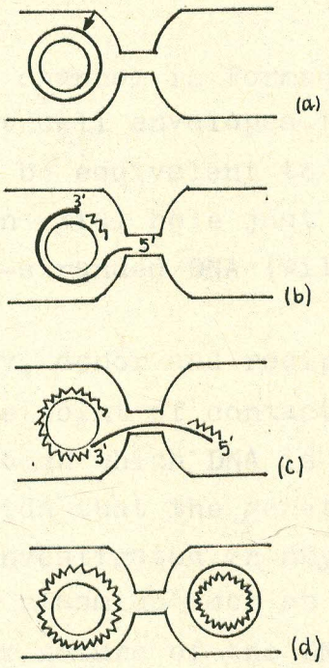



Fig.1. Conjugation between donor and recipient cells.
(a) Formation of mating pair. Nick introduced at origin of transfer in one specific strand, denoted by arrow.

(b) and (c): The nicked strand transferred from 5' end into recipient possibly through the pilus. Replication of single strands in donor and recipient cells (new DNA denoted by jagged line)

(d) Recircularisation of both plasmids. From Williams P.A. 1978.

The pilus is required at least for the cell to cell interaction that precedes DNA transfer; However, it is not known whether it forms the bridge through which DNA passes from the donor to recipient cell. In the case of the F pilus, one of the best studied, the pilus forms a specific contact with the potential recipient cell and its retraction brings the cells into wall to wall contact. The pairs or aggregates thus formed are initially sensitive to shear forces but are then stabilized by an unknown process.

Presumably a channel is formed between the juxtapozed donor and recipient cell envelopes to allow DNA transfer. This channel might be equivalent to a 'stab' of the pilus, which has an axial hole just large enough to accommodate single-stranded DNA (Willets& Wilkins 1984).

Alternatively, donor and recipient cell envelopes fuse locally at the point of contact and a transmembrane pore is formed through which DNA is transferred. It is only in IncF plasmids that the genetics of transfer system have been investigated in any detail; with the exception of a few plasmids such as the ColIb-p9 and P144, the molecular nature of the DNA transmitted by other groups of conjugative plasmids has not been studied and care should be exercised in extrapolating to these the information gained for F (Willets and Wilkins 1984). The genes of F responsible for conjugation are located on a continuous section of the plasmid and are transcribed as a single operon, the tra(transfer) operon (Fig. 2) in which there are 12 structural genes. The first eight of these, tra A to tra H, are responsible for pilus synthesis. Mutations in any of these genes result in cells without pili, which are unable to transfer.

The tra S gene determines the surface exclusion property (not incompatibility) which reduces the ability of a like plasmid to be transferred into the cell. Both traD and traI are involved in the DNA metabolism in the donor during conjugation and traG is bifunctional and mutations in it are defective in both pilus formation and DNA transfer.

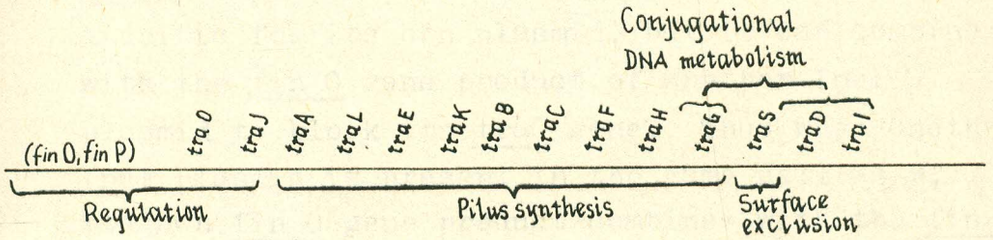



Fig.2: The tra operon of F-like plasmids.
From Williams P.A 1978.

The regulation of the tra operon is very complex and shows some interesting features (Fig.3)

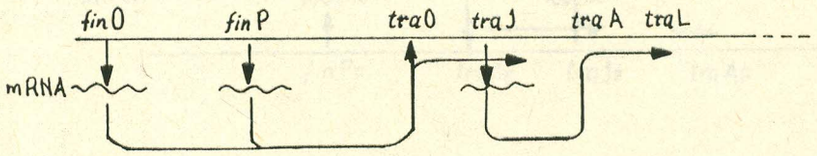



Fig.3: Regulation of tra operon in F-like plasmids; the traJ gene product acts as a positive regulator for the tra operon. The fin O and fin P gene products act together at tra O to block transcription of tra J, so that normally the tra J gene product is not synthesised and the tra operon is repressed. From William P.A. 1978.

This regulatory mechanism accounts for three properties of F and other IncF plasmids.

(1) F itself lacks a functional fin O gene and hence shows derepressed pilus synthesis and transfers at high frequency.

(2) The $fi^+$ character of IncF plasmids which are compatible with F is due to lack of specificity of their fin O gene (Fig.4). Each fin P gene product is specific for its own plasmid, but it can combine with the fin O gene product of another IncF plasmid to block its traO gene. Thus when another IncF plasmid is present in the same cell as F, its own fin O gene product combines with the fin P gene product of F to repress the normally depressed transfer system of F.

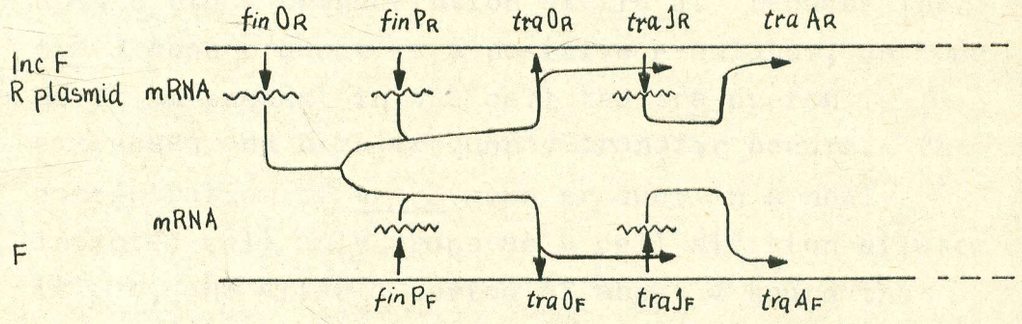



Fig. 4: Fertility inhibition of F through interaction of the regulatory systems of an IncF plasmid and F itself. F has no functional finO gene and consequently expression of the tra operon is normally depressed.

$fi^+$ = fertility inhibition

The fin O gene products of other compatible Inc F plasmids are non-specific: when one of these is present in a cell with F, its fin O product can interact with fin P product of F to repress expression of the F tra operon. From Williams P.A. 1978.

(3) The efficiency of $fi^+$ plasmids in the short term is poor because of the repression of the tra operon. However, if donor and recipient cultures are left together for longer periods, say overnight, an unexpectedly high extent of transfer is obtained. This is because pilus synthesis is not repressed in a newly infected cell, which can further transmit its plasmid at high frequency. The explanation is that in a newly infected cell, there is no preferential expression of either fin O, fin P or tra J genes. Consequently there is some transient synthesis of the tra J gene product and this only slows down and ceases as the fin O and fin P gene products build up and block transcription of tra J. Because the tra J gene product is a positive regulator, as long as it is present in the cell the tra operon is expressed and high frequency transfer occurs. The concentration of tra J gene product in a newly infected cell only drops as a cell division dilutes it out, and after a period of about 4 hours the progeny are once again in the normal repressed low transfer state. This feature is advantageous since it ensures that in a stable population the ability to transfer is repressed with a resultant saving in biosynthesis. When a recipient population becomes available and once some small amount of transfer takes place, the depression of newly infected cells ensures an efficient infectious spread of the plasmid.

There is no genetic relationship between the transfer system of Inc F plasmids and those of other Inc groups, and transfer deficient (tra$^-$) mutants of one group are not complemented by plasmids of another. There are likely to be analogies, however, since, for example, Inc I plasmids also change from being repressed to derepressed in newly infected cells.

## 1.6. PLASMID INCOMPATIBILITY

Plasmid incompatibility is the inability of two different plasmids to coexist stably in the same host cell in the absence of continued selection pressure. One may speak of incompatibility only when it is certain that entry of the second plasmid has taken place, and where DNA restriction is not involved (Novick et al 1976). Groups of plasmids that are mutually incompatible have been variously referred to as incompatibility or as compatibility classes or types. Since the members of such a group are mutually incompatible, it seems more logical to refer to it as an incompatibility group, class or type. The sex factor F (denoting fertility) in E.coli K12 was the first extrachromosomal genetic element recognised, as a result of its ability to carry chromosomal genes with it into a small proportion of recipient during conjugation; this property is shared by many other bacterial plasmids since discovered. The sex factor F has one particular property which distinguishes it from many other plasmids, namely, the ability to integrate covalently into several possible positions in the E.coli chromosome, where it acts as a set of chromosomal genes during replication and cell division. This dual existance, either as an autonomous unit or integrate into the chromosome, has in the past led to it being considered as a rather distinct entity from a plasmid, and it has consequently been referred to as an episome.

It is now more usual to think of F as a plasmid which has an additional phenotypic character, the ability to integrate into the chromosome; this recognises that the similarities between F and other plasmids are greater than between F and bacteriophage λ, which has also been termed an episome because of its ability to exist either autonomously or integrated (Williams P.A 1978). The sex factor F is extremely efficient at promoting its own transfer; in a few hours the number of recipient cells converted to $F^+$ can exceed the number of donor cells. Examination of $F^+$ cells in the electron micrograph shows the presence on the cell surface of organelles, the sex pili, which are not present on $F^-$ cells. These pili play an important part in the mechanics of conjugation, since rapid mixing of an $F^+$ culture shears the pili from cells and removes the donor ability, which is only regained when new pili have been synthesised (Williams, P.A. 1978). Most other plasmids are much less efficient at conjugational transfer, at least in short mating experiments. This is reflected in the lower number of piliated cells in donor cultures. The reason for this is an efficient system for repression of pilus synthesis present on most plasmids, but not on F. It is possible to make mutants in which pilus synthesis is derepressed and the transfer efficiency much increased (drd mutants). Two common types of sex pili are found. Some plasmids specify production of pili very similar in morphology to the F pilus, which are termed F-like. These plasmids have the additional property that, if they can be transferred to a cell containing F, they repress synthesis of the F pilus and drastically reduce the conjugational transfer frequency of F. Plasmid with this phenotype are termed $fi^+$ (fertility inhibition), and include members of all classes found in enteric bacteria, R plasmids, Col plasmids, Hly, Ent and K88.

Other plasmids specify morphologically different pili, which are similar to those found on cells containing Col I plasmids (eg. Colb- P9), and are called I-like. They differ in being shorter than F-like pili, about 2um as against 20 um, and seldom have the central axial hole often seen in F-like pili. They also differ in their antigenicity and in the adsorption of pilus-specificic (or male-specificic) phages. Several phages are specific for F-like pili (eg. F1, F2, fd) and one If1, is specific for I-like pili. Most plasmids which produce I-like pili have no effect on fertility of F if present with F in the same cell and are termed $fi^-$. It was at first thought that fi phenotype could be used as a primary method of grouping plasmids into only two classes, $fi^+$ and $fi^-$ plasmids. As more plasmids were isolated and characterised it became apparent that this was an oversimplification. A more precise method of classification resulted from observations that there are barriers which present the transfer of certain plasmids into a cell containing a different plasmid. This superinfection immunity is the result of two distinct phenomenon. The first is a surface exclusion by a resident plasmid, which does not affect the formation of a mating pair but does inhibit the conjugational transfer of DNA into the cell. Secondly there is incompatibility between plasmids; even if an incompartible plasmid is introduced into a cell arleady containing a plasmid by sufficiently strong selective pressure, the two plasmids interact by inhibiting replication of each other. The net result is that upon removal from any selective pressure which is ensuring that both plasmids coexist, the plasmids rapidly segregate during a few generations to give a mixed population of progeny containing either one or the other plasmid.

The study of incompatibility was extended, in particular by Datta, and is now the major method of classifying plasmids. An incompatibility group is defined by the following experimental criterion. Two plasmids, p1 and p2, are assigned to the same group if a strain, usually E.coli K12, into which both plasmids have been transferred ($p1^+$ or $p2^+$) segregates progeny which are either $p1^+$ or $p2^+$ but no stable ($p1^+$ $p2^+$) lines. If p1 and a third plasmid p3 are similarly shown to be incompatible, then p3 is assigned to the same group and p2 and p3 will also prove incompatible. The various groups are designated Inc groups with a letter characteristic of a group. By this criterion, the $fi^+$ plasmids which specify F-like pili are not a single class, but are made up of five incompatibility groups, designated Inc F1-IncFV. The $fi^-$ plasmids fall into about 14 groups, of which those producing I-like pili constitute about 5. One group which deserves some special mention are the IncP plasmids (eg RP4, Table 1) the original members of which were R plasmids isolated in P.aeruginosa but representatives of which have now been isolated in wide range of genera. This range of parental strains is paralleled by a very wide range of genera into which IncP plasmids can be transferred, and they may prove to be a major vector for transfer of genetic information across wide generic boundries in the wild.

TABLE 1 : PLASMID INCOMPATIBILITY GROUPS

| Plasmid Incompatibility Groups<br>Plasmids of enteric bacteria | | |
|---|---|---|
| Published group designations (including synonyms) | Suggested phenotypic notations | Plasmid prototypes |
| FI | Inc FI | F.R386(HH) |
| FII | Inc FII | R100, R6, R1 |
| FIII | Inc FIII | ColB-K98 |
| FIV | Inc FIV | R124(HH) |
| FV | IncFV | Folac |
| Com5 | Inc5 | R27(IP) |
| M.Com7 | IncM, Inc7 | R446b(HH)<br>R69(IP) |
| Com9 | Inc9 | R71(IP) |
| Com10 | Inc10 | R72(IP) |
| Com11 | Inc11 | R147(IP) |
| A | IncA | RA1(HH) |
| B | IncB | TP113, TP125 |
| C.com6 | IncC, Inc6 | R40a(IP), R55(IP) |
| H | IncH | R27(HH) (=TP117) |
| Ic, Com1, II | Incla, Incl, Incl1 | ColIb-p9, R144(HH) |
| 12 | Incl2 | TP114 |
| ly | Incly | R621a(HH) Collb |
| J | IncJ | R391 (HH) |
| L | IncL | R371a(HH) |
| N.Com 2 | IncN, Inc2 | N3. R15 |
| O | IncO | R, R724(HH) |
| P.Com 4 | Inc P, Inc4 | RP4 |
| S | IncS | R478(HH) |
| T | IncT | Rtsl |
| W | IncW | S-a, R7K |

From Novick et al 1976

(a) This list is not based on up date data.

(b) (HH) and (IP) indicate that the plasmids so designated belong to the Hammersmith Hospital and Institut Pasteur Series, respectively.

(b) These series have overlapping numbers that had not yet been reconciled at the time they were first published.

(c) In some cases synonyms have been listed, because these exist in the literature, and general agreement on a single designation had not yet been reached. In some cases, the indication of synonyms is provisional, since the prototype plasmids had not all been fully tested for compatibility with all of the other prototypes listed at the time.

TABLE II PLASMIDS OF S.AUREUS

| | Plasmids of Staphylococcus aureus | |
|---|---|---|
| Group | Phenotyoe | Plasmid proto-types |
| 1 | Inc1 | pI524, pl258 |
| 2 | Inc2 | pII47 |
| 3 | Inc3 | pT127 |
| 4 | Inc4 | pS177 |
| 5 | Inc5 | pC221 |
| 6 | Inc6 | pK545 |
| 7 | inc7 | pUBlO1 |

pUBlO1 is a plasmid carrying genes for penicillinase and fusidic and resistance present in in strain

From Novick et al 1976

The various incompatibility groups reflect wide differences in genetic structure. The degree of DNA homology between plasmids can be assessed experimentally by two methods, hybridisation and heteroduplex analysis. In hybridisation the fraction of single - stranded DNA from one source which will bind to single - stranded DNA from a second source (i.e. is complementary) is measured. This gives the overall gross homology without specifying its distribution. One procedure is to bind an excess of the purified DNA of one plasmid onto a nitrocellulose membrane, prior to this the DNA is sheared to small fragments by sonication and denatured to dissociate the double strands. The membranes are then incubated with a small amount of sheared, single-stranded DNA of the second plasmid which has been labelled radioactively by growing the host strain on radioactive medium. The fraction of the total radioactivity which becomes bound to the membrane is a measure of the homology between the plasmids since the only DNA which will bind is that which has base sequences complementary to the membrane-fixed fragments. Heteroduplex analysis is far more specific and can detect short regions of homology of only 100-200 base pairs, and locate them on the plasmid. The purified DNA from two plasmids, most of which will be present as open nicked circles, is mixed, denatured to separate the strands and re-annealed. Many of the original complementary strands come together to form homoduplexes, but where there is homology between the plasmids, some heteroduplexes between opposite strands of the two plasmids form (Fig. 5). The renatured mixture is then prepared for electron microscopy and examined. Homoduplexes are seen as circles of thick, smooth curves of double-stranded DNA and single-stranded DNA, which appears thinner and less smoothly curved (Fig. 6). The double stranded sections correspond to homology and the single-stranded sections to non-homology.

Eithty-five percent of the sequence present in R1 are also found on R6. It might be concluded that all three are descendants of the same plasmid, and that the remarkable similarily between R6 and R100 is a result of carriage of one or both of these between Japan and Germany prior to their isolation. If this is so, it demonstrates the immense possibilities for dissemination afforded by an R plasmid (which can be infectious between bacteria) present in bacteria (which are infectious between humans) and magnified by the ease and rapidity of international transport (Williams P.A. 1978).

R1 has resistance determinants to Amp, Cml, Kan, Sul and Str. It was isolated from Salmonella typhimurium (Britain) R6 was isolated from Salmonella typhimurium (German) R100 also called 222 and NR-1 was isolated from Shigella flexneri in Japan.

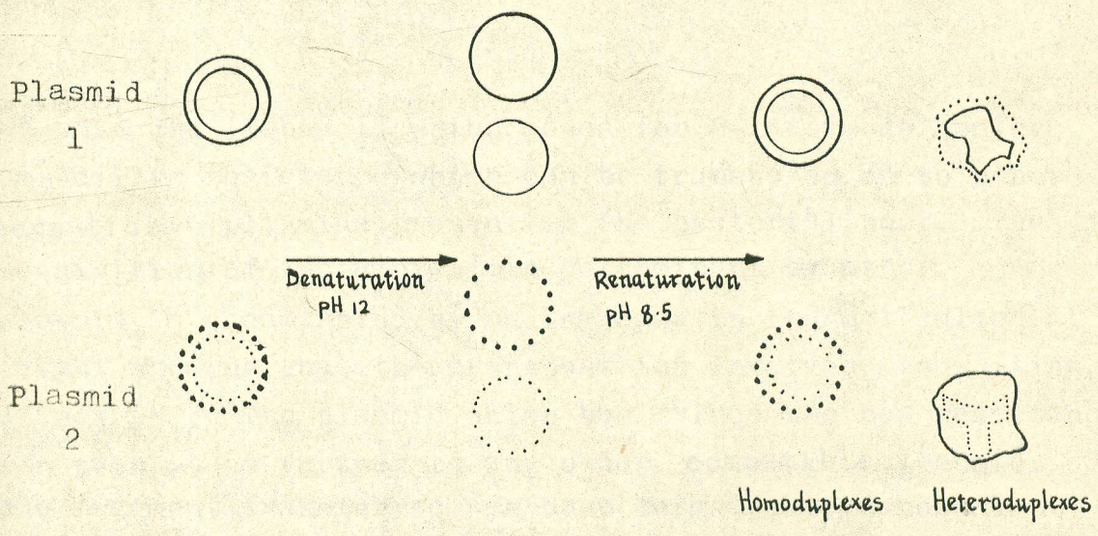



Fig.5 Diagrammatic representation of formation of heteroduplexes. Strand separation of plasmid dublexes only takes place if one or both strands are nicked; due to shearing forces involved in handling, this is almost invariably true for most of the molecules. From Williams P.A. (1978).

HETERODUPLEX MOLECULES

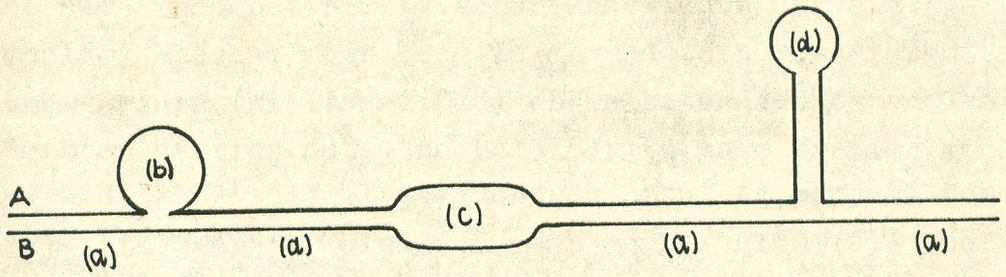



Fig. 6 Structural features seen in heteroduplex molecules. Double-stranded DNA is seen as thicker, less kinked structures than single-stranded DNA. (a) Areas of complete homology between A and B. (b) An I-D loop, resulting from an insertion in A or a deletion in B. (c) Non-homology between A and B. (d) a transposition with terminal inverted repeat sequences, present in A but not in B. From Williams P.A. (1978).

Plasmid RP4 (Table 1) which codes for B-lactamase confers ampicillin resistance which can be transposed on to other compatible plasmids present in the bacterial host. The acquisition of the ampicillin determinant by other plasmid is accompanied by an increase in its molecular weight showing that the transposition involves acquisition of new DNA. The plasmid which has gained the new resistance can pass it on further to any other compatible plasmid. The fragment transferred has been termed a transposon.

Heteroduplex analysis is also a powerful tool for mapping plasmid-genes. Hybridisation has shown that plasmids of the same incompatibility group share considerable DNA homology whereas much less is shared with plasmids of other groups (Williams 1978) where heteroduplex analysis has been carried out, the homology is more precisely defined. F itself (IncFI) shares about 44 percent homology with many $fi^+$ plasmids such as R1, R100 (all IncFII, and therefore compatible with F) and with Col V-K 94 ($fi^-$, Inc FI) and the common area of homology is in one long section of the plasmid molecule. By combining this with genetic mapping it can be shown that this section corresponds only to the genes responsible for conjugational transfer on each plasmid, and not to any of the other functions of either F(eg. chromosomal integration) or of the other plasmid (eg. drug resistance or colicin production). These results imply that the IncF plasmids have evolved as a result of a plasmid with genes capable of coding for its own conjugational transfer (i.e. a sex factor) which, during transfer between strains, is able to acquire other genes which give it additional phenotypic characteristics and thus increase the selective advantage it bestows on the host cells. Heteroduplex comparison amongst R plasmids from different locations can show surprisingly extensive homology. R1, R6 and R100 were originally isolated in England, Germany and Japan, respectively, and are all IncFII plasmids (Table 1). R6 has resistance determinants for chloramphenicol, kanamycin, neomycin, sulphonamides, streptomycin and tetracycline. R100 has resistance determinants for chloramphenicol, sulphonamides, streptomycin and tetracycline. R6 appears to be identical to R100 except for three additional sections which may be partly attributed to its additional resistance to kanamycin and neomycin.

## 1.7 URINARY TRACT INFECTIONS:

About 70 to 80 percent of urinary tract infections are caused by E.coli. (Murdoch et al 1968) and it is generally considered that the source of infecting organisms is the patients own bowel. Mond et al (1965) found 81 percent of infections to be caused by E.coli; the next most common were Proteus and Staphylococcus albus. In patients who have undergone operations on the urinary tract, or who are suffering from recurrent intractable infection, other organisms, particulary Pseudomonas and Klebsiella become important (Spencer et al 1968). Spencer et al (1968) obtained strains of E.coli from patients on a female medical ward who had urinary tract infections. Of 221 strains examined 82 percent were resistant to sulphonamide, 33 percent to tetracycline, 30 percent to ampicillin 10 percent to streptomycin, 4.5 percent to chloramphenicol and 1.5 percent to nitrofurantoin. Similar results were found by Murdoch et al (1968). In hospital patients, therefore, and in patients with recurrent urinary tract infections, antibiotic resistant organisms do appear to be important. However, even in patients seen in general practice antibiotic-resistant organisms may be important at the present time. Brumfitt et al (1971) examined the organisms isolated from 53 women with urinary tract infections seen in general practice, 37 of them were infected with Ecoli and in seven of these (19 percent) the strains were resistant to one or more antibiotics, Resistance was R-factor mediated in six of these. They further investigated the antibiotic resistance of E. coli urinary tract infections in general practice and their relationship to faecal flora.

Of 37 women with E.coli urinary tract infection, seven were infected with antibiotic resistant organisms; resistance was R-factor determined in six. Carriage of predominantly resistant faecal coliform flora was correlated with the development of urinary tract infections with resistant organisms.

Willet (1975) in his paper on "The Survey of Antibiotic sensitivity pattern in Dar es Salaam" - a study done at the Muhimbili hospital on hospitalised, uncatheterised patient with U.T.I. revealed that the resistance to commonly used antibiotics that are used in the treatment of urinary tract infections indeed exists and is high. The incidence was particularly high for penicillins (ie ampicillin), sulphonamide and the tetracyclines.

Although Willet's work did not include community acquired resistance to antibiotics; Nevertheless it suffices to support the idea that resistance to antibiotics exists in Dar es Salaam and is increasing far and wide.

Mfuko (1977) followed the resistance pattern to tetracycline during the years 1970 to 1976 from the available sensitivity records at the Central Pathology Laboratory (C.P.L.) of the Muhimbili Medical Centre (M.M.C.) and revealed the increasing frequency of resistance to the drug. (Table III)

TABLE III: TETRACYCLINE RESISTANCE PATTERN OF URINARY TRACT INFECTIONS 1970 - 1976 MUHIMBILI MEDICAL CENTRE

| YEAR | E.Coli | | Klebsiella spp. | | Proteus spp. | | Pseudo-manads | | Staph aureus | | Strepto-coccus Afaecalis | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B | A | B | A | B |
| 1970 | 695 | 71 | 268 | 57 | 78 | 65 | 59 | 34 | 295 | 35 | 137 | 18 |
| 1971 | 792 | 54 | 363 | 58 | 141 | 36 | 82 | 52 | 238 | 32 | 210 | 16 |
| 1972 | 230 | 60 | 92 | 53 | 29 | 45 | 19 | 47 | 71 | 16 | 85 | 27 |
| 1973 | 222 | 54 | 79 | 57 | 39 | 28 | 9 | 33 | 33 | 7 | 37 | 14 |
| 1974 | 516 | 75 | 210 | 63 | 134 | 70 | 54 | 43 | 119 | 51 | 58 | 48 |
| 1975 | 419 | 80 | 288 | 69 | 88 | 86 | 68 | 63 | 117 | 62 | 107 | 59 |
| 1976 | 596 | 79 | 490 | 73 | 157 | 82 | 103 | 68 | 246 | 71 | 160 | 51 |

Key: A total number of patients with Urinary tract infection showing significant clinical results.
B percentage resistance to tetracycline.

From Mfuko, 1977

Similar retrospective studies were performed by Malisa and Douglas (1977) (unpublished observations) Meselle et al (1980); Kessy and Douglas (1982) unpublished observation Chauya and Douglas (1983) also unpublished. In this case however; all the first-line and second-line drugs used at the Centre were followed.

During the years 1978 and 1979 Maselle et al (1980) observed that approximately 3400 coliforms were isolated from urine specimens investigated at the service wing of the department of Microbiology/Immunology, Muhimbili Medical Centre (M.M.C.) Escherichia coli, Klebsiella-Enterobacter and proteus species were the commonest isolates in that order of frequency. Pseudomonads and other coliforms were also seen. Between 50-90 percent of all coliforms were resistant to ampicillin and tetracycline, the commonly used antibiotics in M.M.C. Resistance was directly proportional to the amounts of drug used. Isolates were generally more susceptible to the less common antimicrobials. Resistance tended to increase with time. Outpatient isolates were generally more suceptible than inpatient isolates especially with the less frequently used drugs.

It was further shown that NO isolates were resistant to gentamycin but 15-60% were resistant to kanamycin and streptomycin. There was a tendency for 1979 isolates to be more resistant than 1978 isolates. Furthermore, nalidixic acid co-trimoxazole and nitrofurantoin were placed high on the preferential list for treating U.T.I. without considering cost and toxicity (excluding infections with Proteus and pseudomonads.

TABLE IVa: RESISTANCE PATTERN OF E.COLI TO FIRST LINE DRUGS AT M.M.C.

| ANTIMICROBIAL AGENT | % UTI ISOLATES RESISTANT | | | | |
|---|---|---|---|---|---|
| | 1979 | 1980 | 1981 | 1982 | 1983 |
| NITROFORANTOIN | 10 | 5 | 12 | 6 | 2 |
| CO-TRIMOXAZOLE | 24 | 25 | 45 | 64 | 29 |
| AMPICILLIN | 70 | 71 | 75 | 70 | 75 |
| TETRACYCLINE | 73 | 81 | 89 | 87 | 81 |
| SULPHONAMIDE | 82 | 81 | 92 | 92 | 29 |

From Maselle et al 1980

TABLE IVb: AMOUNTS OF FIRST LINE DRUGS USED AT MUHIMBILI MEDICAL CENTRE FOR URINARY TRACT INFECTIONS

| ANTIMICROBIAL AGENT | DRUGS USED (KG) | | | |
|---|---|---|---|---|
| | 1979/80 | 1980/81 | 1981/82 | 1982/84 |
| NITROFURANTOIN | 10 | 8 | 12 | 9 |
| COTRIMOXAZOLE | 10 | 70 | 105 | 57 |
| AMPICILLIN | 33 | 58 | 54 | 29 |
| TETRACYCLINE | 1 | 520 | 763 | 737 |
| SULPHONAMIDE | 400 | 225 | 678 | 893 |

From Maselle et al 1980

Table IVa represents the resistance pattern of E.coli isolated from U.T.I. to first-line drugs at Muhimbili Medical Centre for the years 1979 - 83 starting from January to December. Table IVb represents the amounts of first-line drugs used during the Governments financial year, 1979 - 1983 starting from July to June the following year.

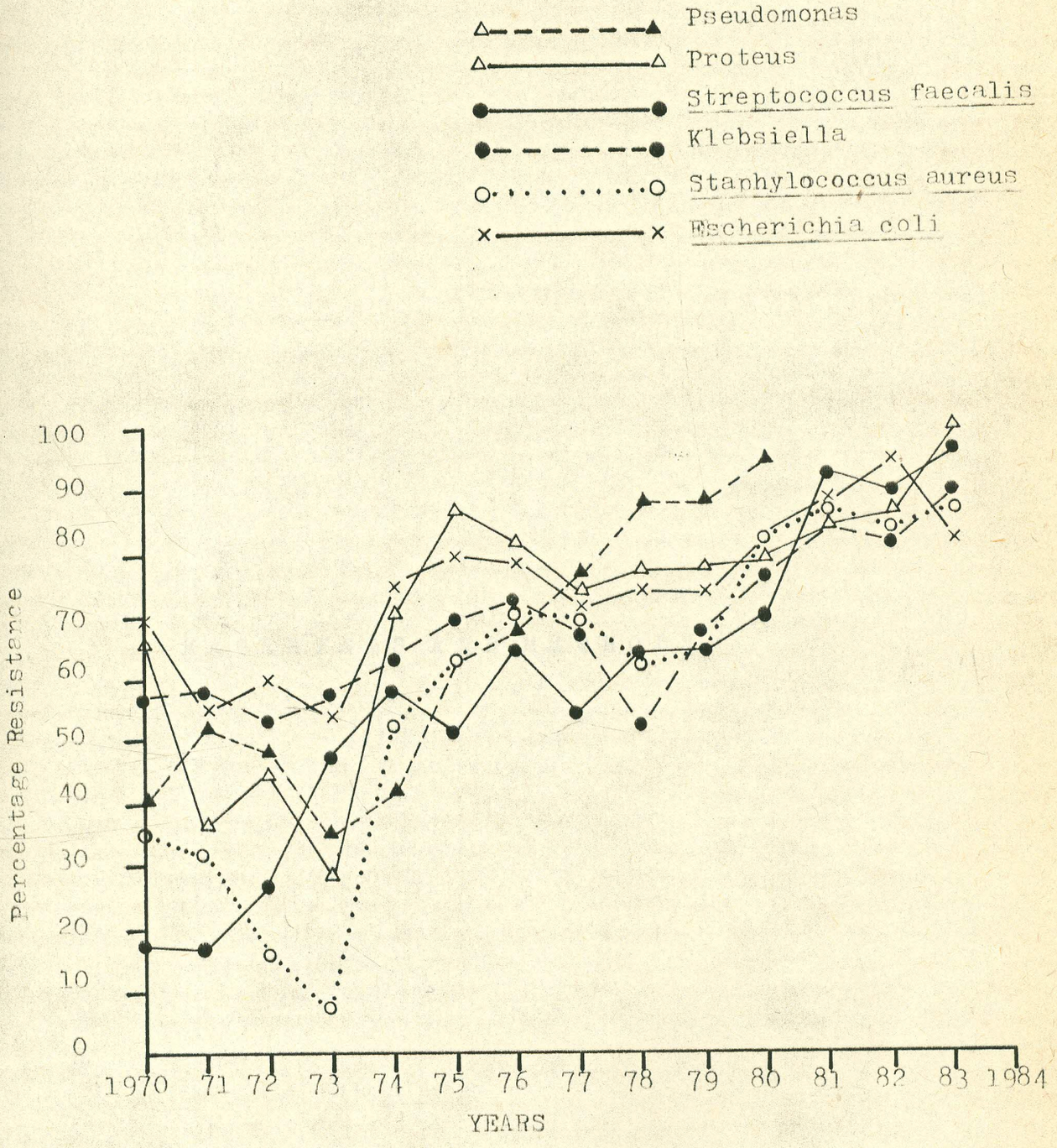
This information is based on Muhimbili Medical Centre records. From Maselle et al (1980)

In 1982 resistance to sulphonamides was very high, but in 1983 fell dramatically from 92 percent to 29 percent although consumption of sulphonamides was increased.

To summarize, the trend of microbial drug resistance as regards urinary tract infections at M.M.C. is on the increase. Although in most cases resistance has been shown to be proportional to the amounts of antibiotics used, there are also several examples where the reverse is also true. For example, the amounts of cotrimoxazole and ampicillin used was nearly halved in 1982/83 compared with the previous year. The percentage of isolates resistant to cotrimoxazole was also halved, but the level of resistance to ampicillin remained the same.

Datta et al (1971) noted that tetracycline has a particular disadvantage in that it exerts strong selection for antibiotic resistant organisms in the faeces. In this respect the resistance pattern of the various organisms to tetracycline in patients with U.T.I. at M.M.C. as from 1970 - 1983 can be used in an attempt to delineate the role of the R-factor in the increase in resistance in M.M.C. Graph 1.

GRAPH I: TETRACYCLINE RESISTANCE FOR VARIOUS BACTERIA ISOLATED FROM PATIENTS WITH U.T.I. IN MMC IN THE YEARS 1970 TO 1983 (%)




Graph based on data collected from Muhimbili Medical Centre Medical Records

# MATERIALS AND METHODS

## 2. MATERIALS AND METHODS

### Cultures

Thirty four (34) stool samples from normal healthy individuals presumably not on antibiotics were collected from students and staff of the University of Aston in Birmingham (U.K.) Another fifty-nine (59) Stool samples were collected from students, staff and other workers of the University of Dar es Salaam, Faculty of Medicine (Tanzania). Subjects in the latter group denied having received antibiotic therapy within the previous three months or more. However, the information with regard to antibiotics therapy may have been unreliable in some cases.

### Media

Oxoid MacConkey Agar No.2 (MCA)
Oxoid Nutrient Agar (N.A)
Oxoid Nutrient Broth (N.B)
Oxoid Urea Agar Base
Oxoid Triple Sugar Agar (T.S.I)
Oxoid Diagnostic Sensitivity Agar (DST)
Oxoid MRVP media (MRVP)
Oxoid Pepton
Oxoid Salmonella-Shigella Agar (modified) (SS Agar)
Oxoid Koser citrate medium
Oxoid Kovacs reagent
Neo-sensitabs.

All stool samples were streaked on MCA No.2 so as to isolate the different types of colonies as well as to differentiate them on the basis of lactose fermentation.

Preliminary identification of the organisms was done as follows:

Lactose positive (Lac+) Colonies:

: not Slimy - Escherichia
: Slimy - Klebsiella
: Other - Enterobacter spp.

Lactose negative (lac-) colonies:

: Swarming - Proteus
: green - Pseudomonas
: pink - Serratia
: Other - Salmonella/Shigella.

Since rapid identification methods were not available, coupled with the fact that reagents and media were difficult to acquire when needed due to financial constraints, no effort was made to identify the organisms further. Prolonged storage of the cultures might result in R-factor mediated resistance properties being spontaneously eliminated. Cultures were stores in Nutrient Agar (N.A.) slopes in bijou bottles after inoculation from single colonies for storage.

ANTIBIOTICS SENSITIVITY TESTING:

Antibiotic sensitivity testing was accomplished by a quantitative neo-sensitab diffusion method using oxoid diagnostic sensitivity agar (DST). The diffusible amount of the various antibiotics are as shown in Table V. Plates were dried for twenty to thirty minutes at 37°C before use.

TABLE V: DIFFUSIBLE AMOUNTS OF THE ANTIMICROBIALS CONTAINED IN THE NEOSENSITAB TABLETS USED FOR SENSITIVITY TESTING

| Antimicrobial | Abbreviation | Diffusible amount of antimicrobial in mcg |
|---|---|---|
| Ampicillin | Amp | 33 |
| Carbenicillin | Car | 115 |
| Streptomycin | Str | 100 |
| Kanamycin | Kan | 100 |
| Gentamycin | Gen | 40 |
| Tetracycline | Tet | 80 |
| Chloramphenicol | Chl | 60 |
| Trimethoprim | Tri | 5.2 |
| Trimethoprim + Sulfa | Cot | 240 + 5.2 |
| Nitrofurantoin | Nit | 260 |
| Nalidixic acid | Nal | 130 |
| Sulphonamide | Sul | 240 |

A $10^{-2}$ dilution of an overnight (ON) culture of the organisms grown at 37°C in oxoid NB was used to flood the plates. The excess fluid culture was removed from the plates using sterile Pasteur pipettes. The Neosensitabs were then placed firmly on the seeded plates to prevent the tablets from falling off during incubation.

All plates were left on the bench for two hours to allow for antibiotic diffusion, and incubated ON at 37°C. The diameters of the zones of inhibition were measured and recorded.

Before sensitivity testing, care was taken not to store the cultures longer than seven days.

TRANSFER EXPERIMENTS:

The term donor and recipient cultures are used here to mean the strains being tested in those roles, irrespective of whether R-transfer takes place. The recipient should have at least two recognisable differencies from the donor. One which allows it to be separated from the donor on a selective medium, the other, a stable marker, by which it can be distinguished from mutants of the donor. The marker can be a chromosomal drug resistance absent in the donor culture, for example nalidixic acid or streptomycin resistance. Maker may also be lactose fermentation or its lack, or a second drug resistance trait not used for selection but only for identification. Escherichia coli K12 strains are most often used as recipients when the donor is a Gram-negative bacillus. Lactose-fermenting strains are convenient donors when the recipient is a non-lactose fermenting and vice versa. (Datta, N. 1978).

Donor strains used in transfer experiment were as follows:

(a) Escherichia coli K12 W3110 containing R-plasmid RP1 supplied by Mr. El-Haffar, a Ph.D. student (1983) at the Department of Pharmacy, University of Aston in Birmingham, B4 7ET U.K.

(b) Isolates from the stool samples referred to in this thesis as "unknowns".

(c) Escherichia coli isolates from urine samples of patients suffering from urinary tract infection (U.T.I.) Supplied by Mr. E.L. Msafiri of the Department of Pharmaceutics/Pharmaceutical Microbiology, University of Dar es Salaam, Faculty of Medicine.

Recipient strains used in transfer experiments were:

(a) Escherichia coli K12, plasmid free streptomycin resistant mutant ($lac^-$; $R^-$; $Str^R$)*.

(b) Escherichia coli K 12, plasmid free nalidixic acid resistant mutant ($Lac^+$; $R^-$ $Nal^R$)* The above two strains were kindly supplied by Dr. Ruth M.M. Klemperer of the Department of Pharmacy, University of Aston in Birmingham B4 7ET (U.K.).

(c) As donors (b) above provided they were sensitive to the particular antibiotic used in transfer (unknown recipients).

Preliminary work was demonstrated that Plasmid RP1 could be transferred from E.coli K12 W3110 (RP1) (known donor) to E.coli K 12S and E.coli K12N (known recipients with chromosomal non-transferrable resistances).

Preparation of mating cultures

A loopful of donor and recipient was innoculated into N.B. in separate universal bottles and grown ON. One ml was taken from each and innoculated separately in 9ml of N.B. These cultures were allowed to grow in a shaking water-bath at 37°C to logarithmic phase and their optical density adjusted approximately to 0.5 at 470 n.m.

*Recipient phenotype ($R^-$) without an R plasmid
$Str^R$ and $Nal^R$ - chromosomally coded streptomycin and nalidixic acid resistance respectively.

The mating mixture was prepared by taking 0.1 ml of donor and 0.5 ml of recipient to 10 ml of fresh N.B. contained in a 50ml Erlenmeyer flask. The mating mixtures were incubated overnight at either 28°C or 37°C and in a shaking or stationary waterbath. This was in order to achieve the most efficient transfer and to control for potentially temperature sensitive transfer mechanisms in donor strains.

After ON incubation $10^{-4}$ and $10^{-6}$ dilutions of the mating mixture were prepared using an Epperndolf micropipette. Seven drops per plate of each dilution were plated out on MCA no.2 containing the appropriate antibiotics. For example, if the recipient was E.coli K12S, and the donor was $lac^+$ then only one antibiotic ie. tetracycline 25mcgml$^{-1}$ was used and transcipients selected on the basis of lactose fermentation (lactose negative colonies growing on tetracycline). If however, the recipient was E.coli K12N, then transcipients were selected with tetracycline 25mg ml$^{-1}$ and donors counter selected with nalidixic acid 25mg ml$^{-1}$. After preliminary tests, the $10^{-6}$ dilution yielded better results. Hence these plates were dublicated. All plates were incubated ON at 28°C and 37°C. Colonies appearing on the selective media were purified on the same medium and their drug susceptibility tested. The drugs and concentrations used are as shown in table VI.

TABLE VI: FINAL CONCENTRATIONS OF THE VARIOUS ANTIMICROBIALS USED FOR SELECTION OF TRANSCIPIENTS

| Antimicrobial agent | Final Concentration mcg/ml | Manufacturer |
|---|---|---|
| Nitrofurantoin | 50 | SIGMA N-7878 LOT 276-0275 |
| Carbenicillin | 30 | BEECHAM RESEARCH LABORATORIES T.S.A. NO. 305-9723(1) |
| Tetracycline | 25 | SHANGAI PHARMA-CEUTICALS LOT 78047902 |
| Ampicillin | 25 | FARMITALIA-CARLO ERBA LOT. 2580 |
| Nalidixic Acid | 25 | BOEHRINGER MANNHEIM GmbH 1479208 |
| Streptomycin | 20 | Madexport-U.S.S.R. Lot 1550383 |
| Chloramphenicol | 25 | LAGAP SA SWITZER-LAND LOT 90114 |
| Kanamycin | 20 | SHANGAI PHARMA-CEUTICALS LOT 310072 |

Before incorporation, all antibiotic solutions were sterilized by bacterial filtration using satorius membrane filters of pore size 0.2 µ and 25 mm diameter in swinnex membrane holders with the aid of sterile 10ml syringes.

Positive and negative controls were set to ascertain antibiotic activity, sensitivity to the antibiotics and viability. Transfer experiments were done by a method suggested to the author by Dr. Ruth Klemperer. Only minor modifications were made.

The same procedure was repeated using the collected samples. An attempt was also made to transfer drug resistance from "Unknown" donors to Unknown recipients.

Biochemical differentiation of the bacteria was done as described by Buchanan et al (1974)(Appendix IVa to IVc). Sub-classification of the bacteria beyond the genus was not done.

# RESULTS

3. RESULTS

TABLE VII: ISOLATION FREQUENCY OF ESCHERICHIA KLEBSIELLA AND PROTEUS ISOLATES FROM STOOL SAMPLES FROM HEALTHY HUMAN SUBJECTS

(%)

| | Dar es Salaam | Birmingham |
|---|---|---|
| Escherichia isolates | 37.3 | 88.2 |
| Klebsiella isolates | 39.0 | 11.2 |
| Proteus isolates | 23.7 | 0.0 |
| Total | 100 | 100 |

Table VII is a Summary of the identification of the organisms isolated from both Dar es Salaam and Birmingham samples. The biochemical identification was done as described by Buchanan et al 1974 and is shown in appendix IVa and IVc.

TABLE VIII: INCIDENCE OF DRUG-RESISTANT BACTERIA ISOLATED FROM HEALTHY HUMAN SUBJECTS

| | Dar es Salaam | Birmingham |
|---|---|---|
| Number of Isolates examined | 59 | 34 |
| Percentage resistant to: Tetracycline | 41 | 21 |
| Ampicillin | 24 | 29 |
| Streptomycin | 17 | 50 |
| Chloramphenicol | 14 | 3 |
| Sulphonamides | N.D. | 77 |
| Nalidixic acid | 2 | 0 |
| Nitrofurantoin | 11 | 0 |
| Gentamycin | 0 | 0 |
| Kanamycin | 9 | 3 |
| Carbenicillin | 49 | 3 |
| Cotrimoxazole | N.D. | 0 |
| Trimethoprim | N.D. | 1 |

Table VIII shows percentage resistance of isolates to various antibiotics isolated from Dar es Salaam and from Birmingham.

As can be seen resistance to streptomycin and sulphonamides was high in the Birmingham samples while resistance to tetracycline and carbenicillin was higher in isolates from Dar es Salaam. Sulphonamide sensitivity was not done for Dar es Salaam samples because of shortage of neosensitabs. Attempts to use laboratory prepared sulphonamide disks were unsuccesiful.

TABLE IX: RESISTANCE PATTERN OF BACTERIA ISOLATED FROM HEALTHY HUMAN SUBJECTS

%

| Resistance | Dar es Salaam | Birmingham |
|---|---|---|
| Single | 29 | 21 |
| Double | 24 | 38 |
| Triple | 15 | 14 |
| Quadruple | 2 | 9 |
| Quintuple | 2 | 3 |
| Sextuple | 5 | 0.0 |
| S T T | 20 | 14 |

Table IX shows the percentage of isolates resistant to one or more antibiotics for both Dar es Salaam and Birmingham isolates.

Note: In both cities 24% of the samples were resistant to 3 or more antibiotics.

S T T Sensitive to all drugs tested.

TABLE X: INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT ESCHERICHIA ISOLATED FROM HEALTHY HUMAN SUBJECTS (BIRMINGHAM)

| Isolation Number | Resistance | Resistance transferred |
|---|---|---|
| ST 3 | Amp; Str | Amp |
| ST 4 | Chl; Tet; Sul | Tet |
| ST 5 | Sul | ND |
| ST 6 | Str; Sul | NIL |
| ST 7 | Str; Sul | NIL |
| ST 9 | Kan; Sul | NIL |
| ST 10 | Sul | ND |
| ST 11 | Sul | ND |
| ST 12 | Str; Sul | NIL |
| ST 13 | Str; Sul | NIL |
| ST 14 | Amp; Sul | NIL |
| ST 15 | Amp; Str; Tet; Sul | Amp; Tet |
| ST 16 | Str | NIL |
| ST 17 | Car; Amp; Str; Tet; Sul | Tet; Amp |
| ST 18 | Str; Sul | NIL |
| ST 19 | Tet; Sul | NIL |
| ST 20 | Sul | ND |
| ST 21 | Str; Sul; Cot | NIL |
| ST 22 | Amp; Str; Tet; Sul. | Amp; Tet |
| ST 23 | Amp; Sul | NIL |
| ST 24 | Sul | ND |

Table X continued

TABLE X continued ....

| Isolation Number | Resistance | Resistance Transferred |
|---|---|---|
| ST 25 | Str; Tet | NIL |
| ST 26 | Str; Tet; Sul | Tet |
| ST 27 | Amp; Str; Sul | NIL |
| ST 32 | Amp; Tet; Sul | Amp; Tet |
| ST 33 | Str; Sul | NIL |

TABLE XI: SUMMARY OF THE INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT ESCHERICHIA ISOLATES (BIRMINGHAM)

| Resistance of Donor | Number Tested | Number Transferred | Resistance Transferred |
|---|---|---|---|
| Str | 1 | 0 | - |
| Amp Str | 1 | 1 | Amp (1) |
| Str Tet | 1 | 0 | - |
| Str Sul | 6 | 0 | - |
| Kan Sul | 1 | 0 | - |
| Amp Sul | 2 | 0 | - |
| Tet Sul | 1 | 0 | - |
| Amp Tet Sul | 1 | 1 | Amp Tet (1) |
| Amp Str Sul | 1 | 0 | - |
| Str Tet Sul | 1 | 1 | Tet (1) |
| Chl Tet Sul | 1 | 1 | Tet (1) |
| Str Sul Cot | 1 | 0 | - |
| Amp Str Tet Sul | 2 | 2 | Amp Tet (2) |
| Car Amp Str Tet Sul | 1 | 1 | Tet Amp (1) |

Number in parentheses indicate number of strains which transferred resistance.

TABLE XII INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT KLEBSIELLA ISOLATES (BIRMINGHAM)

| Isolation Number | Resistance | Resistance transferred |
|---|---|---|
| ST 29 | Amp; Str; Tet; Sul | Tet; Amp |
| ST 30 | Sul | NIL |
| ST 31 | Amp; Str; | NIL |

TABLE XIII: SUMMARY OF THE INCIDENCE OF TRANSFERABIE DRUG RESISTANCE FROM RESISTANT KLEBSIELLA ISOLATES (BIRMINGHAM)

| Resistance of Donor | Number Tested | Number Transferring | Resistance Transferred to E.Coli K12 |
|---|---|---|---|
| Birmingham | | | |
| Sul | 1 | 0 | - |
| Amp Str | 1 | 0 | - |
| Amp Str Tet Sul | 1 | 1 | Tet Amp (1) |

Numbers in parentheses indicate number of strains which transferred resistance.

TABLE XIV: INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT ESCHERICHIA ISOLATED FROM HEALTHY HUMAN SUBJECTS (DAR ES SALAAM)

| Isolation Number | Resistance of Donor | Resistance Transferred |
|---|---|---|
| X10 | Tet | Tet |
| X11a | Amp | NIL |
| X12 | Tet; Amp | Tet |
| X15 | Tet; Amp; Carb | Tet; Amp |
| X16 | Tet; Str; Car | Tet; Str |
| X20 | Car | NIL |
| X22a | Tet; Car | Tet |
| X29 | Tet; Amp; Chl; Car | Tet; Amp |
| X32 | Tet; Car | Tet |
| K15 | Tet; Amp; Chl; Str; Kan; Car | Tet; Str; Kan |
| TZ3(1) | Tet; Amp; Str | NIL |
| K1 | Tet; Str; Kan | NIL |

TABLE XV: SUMMARY OF THE INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT ESCHERICHIA ISOLATES (DAR ES SALAAM)

| Resistance of Donor | Number Tested | Number Transferring | Resistance Transferred to E.coli K12 |
|---|---|---|---|
| Tet | 1 | 1 | Tet (1) |
| Amp | 1 | 0 | - |
| Car | 1 | 0 | - |
| Tet, Amp | 1 | 1 | Tet (1) |
| Tet, Car | 2 | 2 | Tet (2) |
| Tet, Amp, Car | 1 | 1 | Tet Amp (1) |
| Tet, Str, Str | 1 | 0 | - |
| Tet, Str, Car | 1 | 0 | - |
| Tet, Str, Kan | 1 | 0 | - |
| Tet, Amp, Chl Car | 1 | 1 | Tet Amp (1) |
| Tet, Amp, Chl, Str, Kan, Car | 1 | 1 | Tet Str Kan (1) |

Numbers in parentheses idicate number of strains which transferred resistance.

TABLE XVI: INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT PROTEUS ISOLATED FROM HEALTHY HUMAN SUBJECTS (DAR ES SALAAM)

| Isolation Number | Resistance of Donor | Resistance Transferred To *E.coli* K12 |
|---|---|---|
| TZ4 | Amp; Chl; Nit | NIL |
| K17C | Tet; Nit | Tet |
| K7 | Tet; Amp; Chl | Tet; Amp; Chl |
| X1 | Tet; Nit; Kan | Tet |
| X14 | Tet; Nit | Tet |
| X18 | Chr; Nit | NIL |
| X19 | Car | NIL |
| X21a | Tet | NIL |
| X22 | Car | NIL |
| X24 | Car | Car |
| X27 | Amp; Car | NIL |
| X30 | Car | Car |

TABLE XVII: SUMMARY OF THE INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT PROTEUS ISOLATES (DAR ES SALAAM)

| Resistance of Donor | Number Tested | Number Transferring | Resistance Transferred To E.coli K12 |
|---|---|---|---|
| Car | 4 | 2 | Car (2) |
| Tet | 1 | 0 | - |
| Amp Car | 1 | 0 | - |
| Car Nit | 1 | 0 | - |
| Tet Nit | 2 | 2 | Tet (2) |
| Tet Nit Kan | 1 | 1 | Tet (1) |
| Tet Amp Chl | 1 | 1 | Tet Amp Chl (1) |
| Amp Chl Nit | 1 | 0 | - |

Numbers in parentheses indicate number of strains which transferred resistance.

TABLE XVIII: INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT KLEBSIELLA ISOLATED FROM HEALTHY HUMAN SUBJECTS (DAR ES SALAAM)

| Isolation Number | Resistance of Donor | Resistance Transferred To E.coli K12 |
|---|---|---|
| $K_{10}b_2$ | Tet | NIL |
| T23 | Car | NIL |
| $K_2a$ | Amp; Car | NIL |
| $K_1b$ | Tet; Amp; Chl; Str; Kan; Car | Tet; Str; Kan |
| $K_{10}b_1$ | Tet; Str | Tet |
| K11C | Amp; Car | NIL |
| K2 | Tet; Nit | NIL |
| X2 | Tet; Amp; Chl; Str; Kan; Car | Tet; Amp; Chl |
| X3 | Amp; Car; | NIL |
| X4a | Nal | NIL |
| X6 | Car | NIL |
| X7 | Amp | NIL |
| X9 | Car | NIL |
| X12a | Tet | Tet |
| X17 | Tet; Car | NIL |
| X21 | Nit; Car | NIL |
| X23 | Car | NIL |
| X24a | Tet; Str; Car | Tet; Str |
| X25 | Car | NIL |
| X26 | Tet; Amp; Chl; Str; Car | Tet; Amp |
| X28 | Car | NIL |
| X31 | Car | NIL |

TABLE XIX: SUMMARY OF THE INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT KLEBSIELLA ISOLATES (DAR ES SALAAM)

| Resistance of Donor | Number Tested | Number Transferring | Resistance Transferred To E.coli K12 |
|---|---|---|---|
| Tet | 2 | 1 | Tet (1) |
| Car | 7 | 0 | - |
| Nal | 1 | 0 | - |
| Amp | 1 | 0 | - |
| Tet Str | 1 | 1 | Tet (1) |
| Tet Nit | 1 | 0 | - |
| Tet Car | 1 | 0 | - |
| Nit Car | 1 | 0 | - |
| Tet Str Car | 1 | 1 | Tet Str (1) |
| Tet Amp Chl Str Car | 1 | 1 | Tet Amp (1) |
| Tet Amp Chl Str Kan Car | 2 | 2 | Tet Str Kan (1)<br>Chl Tet Amp (1) |

Numbers in parentheses indicate number of strains which transferred resistance.

TABLE XX: INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT ESCHERICHIA COLI ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION (U.T.I.) (DAR ES SALAAM)

| Isolation Number | Resistance | Resistance Transferred To E.coli K12 |
|---|---|---|
| M1 | Tet; Amp; Str; Nal | Tet; Amp |
| M2 | Tet; Amp | Tet |
| M3 | Tet; Chl; Nit | NIL |
| M4 | Tet; Amp; Str; Nit | Tet; Amp; Str |
| M5 | Tet; Amp; Nal; Car | NIL |

TABLE XXI: SUMMARY OF THE INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM RESISTANT ESCHERICHIA COLI ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION (U.T.I.) DAR ES SALAAM

| Resistance of Donor | Number Tested | Number Transferring | Resistance Transferred To E.coli K12 |
|---|---|---|---|
| Tet Amp | 1 | 1 | Tet (1) |
| Tet Chl Nit | 1 | 0 | - |
| Tet Amp Str Nal | 1 | 1 | Tet Amp (1) |
| Tet Amp Str Nit | 1 | 1 | Tet Amp Str |
| Tet Amp Car Nal | 1 | 0 | - |

Numbers in parentheses indicate number of strains which transferred resistance.

TABLE XXII: INCIDENCE OF TRANSFERABLE DRUG RESISTANCE FROM "UNKNOWN DONORS" TO "UNKNOWN RECIPIENTS ISOLATED FROM HEALTHY HUMAN SUBJECTS (DAR ES SALAAM)

| Unknown Donor | Resistance of Donor | Unknown Recipient | Resistance of Recipient | Resistance Transferred |
|---|---|---|---|---|
| X10 | Tet | K17b | SA | NIL |
| | | TZ4 | SA | NIL |
| | | K10 | SA | NIL |
| X11a | Amp | K17b | SA | NIL |
| | | TZ4 | SA | NIL |
| | | K10 | SA | NIL |
| X15 | Tet; Amp Car | K17b | SA | NIL |
| | | TZ4 | SA | NIL |
| | | K10 | SA | NIL |
| X16 | Tet; Str; Car | K17B | SA | NIL |
| | | TZ4 | SA | NIL |
| | | K10 | SA | NIL |
| X29 | Tet; Amp, Car | K17B | SA | NIL |
| | | TZ4 | SA | NIL |
| | | K10 | SA | NIL |
| K10b2 | Tet | K17b | SA | Tet |
| | | TZ4 | SA | NIL |
| | | K10 | SA | NIL |
| K15 | Tet; Amp Chl; Str Kan, Car | K17b | SA | Str |
| | | TZ4 | SA | NIL |
| | | K10 | SA | NIL |

TABLE XXII CONTINUED

Table XXII continued.....

| Unknown Donor | Resistance of Donor | Unknown Recipient | Resistance of Recipient | Resistance Transferred |
|---|---|---|---|---|
| X1 | Tet; Nit; Kan | X4 | SA | NIL |
| | | X5 | SA | NIL |
| | | X8 | SA | NIL |
| | | X11 | SA | NIL |
| | | X33 | SA | NIL |
| X14 | Tet; Nit | X4 | SA | NIL |
| | | X5 | SA | NIL |
| | | X8 | SA | NIL |
| | | X11 | SA | NIL |
| | | X33 | SA | NIL |
| X13 | Chl; Nit Car | X4 | SA | NIL |
| | | X5 | SA | NIL |
| | | X8 | SA | NIL |
| | | X11 | SA | NIL |
| | | X33 | SA | NIL |
| K7 | Tet; Amp Chl | X4 | SA | NIL |
| | | X5 | SA | NIL |
| | | X8 | SA | NIL |
| | | X11 | SA | NIL |
| | | X33 | SA | NIL |
| K17C | Tet; Nit | X4 | SA | NIL |
| | | X5 | SA | NIL |
| | | X8 | SA | NIL |
| | | X11 | SA | NIL |
| | | X33 | SA | NIL |

SA = Sensitive to the drugs to which donors are sensitive.

# DISCUSSION

## 4. DISCUSSION:

### 4.1 DRUG RESISTANCE

The results of this study show that there were more Escherichia isolated from Birmingham samples while Klebsiella and Proteus isolates were predominant in Dar es Salaam samples (Table VII). No Proteus were isolated from Birmingham samples. The basis for this apparent variability among these two groups is not very clear. However, the lack of sugar tests has made the bacterial identification incomplete. It is quite possible therefore that a few isolates might have been wrongly identified. For example, while Klebsiella species are mostly indole negative, indole positive species are also encountered. Of 168 isolates of K. pneumoniae tested Nishida et al (1979) found that 18% were indole positive and 82% were indole negative and a significant difference in antibiotic susceptibility was found.

The level of resistance as shown in table VIII, was found to be quite high for both the Birmingham and Dar es Salaam samples than observed by other workers (Smith 1966; Spencer 1968) in diseased human subjects. For the Dar es Salaam isolates, the order of resistance carbenicillin > tetracycline > ampicillin > streptomycin chloramphenicol > nitrofurantoin > kanamycin > nalidixic acid. The high level of resistance to carbenicillin is surprising as this, being a parenteral preparation, is not commonly available to the general population. There is therefore a need to study the source of this high resistance.

The order of resistance for Birmingham isolates sulphonamides > streptomycin > ampicillin > tetracycline > chloramphenicol, kanamycin, carbenicillin, trimethoprim. All isolates from Birmingham samples were sensitive to nalidixic acid, nitrofurantoin, cotrimoxazole and gentamycin but in Dar es Salaam gentamycin was the only antibiotic to which all isolates were sensitive.

The percentage of isolates resistant to one antibiotic in Dar es Salaam samples was found to be higher than observed in Birmingham samples (Table IX). The high percentage observed was due to tetracycline or carbenicillin resistance. Since tetracycline is one of the greatly misused drugs in Dar es Salaam, resistance to the drug is therefore very obvious . As suggested earlier, it is not easy to explain the high level of resistance to carbenicillin.

As noted on table IX, there is a high percentage of isolates with resistance to more than 3 drugs (24% or more). This is a situation which is not very encouraging. What may be worth even more thought, is the presence in Dar es Salaam of people with organisms resistant of six drugs (5%). This sextuple resistance was for Tet; Amp; Chl; Str; Kan and Car (Table IX). Even though antibiotics may be more readily available in the U.K., there control may be better i.e. obtainable only by prescription. In Dar es Salaam, as in many other developing countries, there may be more indiscriminate use. Cameroon is one of the examples where antibiotics are sold in open markets without prescriptions (Oxfarm Film Series).

Isolates from Birmingham showed a high percentage of resistance to four drugs. (Table IX). The quadruple resistance was Amp; Str; Tet and Sul. The particular resistance pattern was repeated in all the isolates, suggesting a common plasmid.

Double resistance was also observed to be higher in Birmingham. In most cases the resistance was Str and Sul. This might suggest a common plasmid too. The high percentage of isolates resistant to these two drugs is not only surprising but also questionable. Whether it represents wrong prescribing habits is something that cannot be explained with certainty, unless, a follow up is made by interviewing the patients.

The incidence and degree of antimicrobial resistance has also been studied by Datta et al (1971). She found that there was a significant number of persons excreting multiple resistance bacteria. In some cases a high proportion (5-100%) of total aerobic flora was resistant to one or more antimicrobials. Linton et al (1972) also reported similar findings. The level of resistance found in this study agree very well with those of Datta and Linton above. Skinner and Carr (1974) proposed that one source of these multiple resistance bacteria in human intestinal tract may be associated with food producing animals.

In the U.S.A. at least, it has been found out that the level of antibiotic resistant bacteria in livestock raised for food products to be relatively high. Animals such as cattle, swine, turkey and chickens commonly received antibiotics in levels from 20-50p.p.m. as feed suppliments. Cattle and swine purchased in the State of Missouri for research purposes for example, possessed high level of antibiotic resistant E.coli. Some of these 50-100% had multiple resistance (Skinner and Carr 1974). Mercer et al (1971) suggested that the proportion of enteric flora resistant to antibiotics is thought to be related to the exposure of individuals involved. Among other reasons, such as non-compliance and wrong prescribing habits, it seems logical to suggest that the level of antibiotic resistance observed in solates from Birmingham samples might be due to the levels of resistant bacteria in livestock raised for food products.

In Dar es Salaam however, the main reason for the high level of antibiotic resistance could be due to indiscriminate use of antibiotics. Examples of such indiscriminate use include the use of various antibiotics for chemoprophylaxis against infections such as gonorrhoea.

Antibiotic capsules for example, known as "Missiles" by educated people and "rangi mbili" by the common man (a drug with two colours) are believed to cure all infections. Many people use procain penicillin 'G' oily (PAM) as eye suspension for infections of the eye (Personal experience). Others use various antibiotic powders including penicillin for local application to wound infections. The use of dihydrostreptomycin to check diarrhoea especially in children is not uncommon (personal experience). There are therefore various ways in which antibiotics are used indiscriminately. The effects of antibiotic administration and percentage resistance bacteria in faeces has been studied by Ahart et al (1978).

## 4.2 TRANSFER OF DRUG RESISTANCE FACTORS:

The discovery of infectious drug resistance in Japan about twenty five years ago, has led to studies attempting to delineate the role of resistance factors with the increasing incidence of multiple drug resistance in Gram-negative organisms. It is obvious from the data obtained as well as from other reports (Smith and Armour 1966; Chau et al 1982) that multiple drug resistance is often due to demonstrable R-factors. However, it should be borne in mind also that if antibiotic resistance is transferred in mixed culture from donor to a suitable recipient, this is strong evidence on the basis of previous examples that the resistance is plasmid mediated. It is however, not conclusive proof since chromosomal genes may be transferred by conjugation (Datta 1978).

In this study, a striking difference was observed in the transfer ability of R-factors between isolates from Birmingham samples and those from Dar es Salaam.

Of the (21) Escherichia isolates from Birmingham, only one third were able to transfer their resistances and of the (3) Klebsiella isolates, (1) transferred resistance to E.coli K12S (Tables X,XI, XII and XIII). On the other hand isolates from Dar es Salaam samples transferred resistance at a higher rate with more than half of Escherichia, a half of Proteus and (4) of Klebsiella isolates transferring resistance to E.coli K12S and E.coli K12N. Three out of (5) of E.coli isolated from patients with U.T.I. also transferred resistance to E.coli K12S (Tables XX and XXI).

With regard to "Unknown" donors transferring drug resistance to "Unknown" recipients, transfer ability was extremely low (Table XXII). It seems possible that most of the "Unknown" recipients were either potentially donors rather than recipients, or there was incompatibility between plasmids.

This study also revealed that there was no visually observable difference in the transfer ability for broth matings at 28°C and those at 37°C. However, there were more transcipients when the mating mixtures were left stationary than when shaking was introduced.

The latter fact suggests that shaking overnight decreases the probability of contact, perhaps due to demixing especially when donor and recipient are of different sizes. Shaking is usually aimed at mixing the mating mixtures. The machanics of mixing aim for randomized mixing because a uniform mosaic (chess-board arrangement) is impossible even with the best mixing machine. Random mixing has its time limit. The mechanics of mixing are summarized by Shotton and Ridgeway (1974). Datta (1978) maintains also that the mating mixture be incubated without shaking at 37°C. However, Wiletts and Wilkins (1984) had the opinion that during shaking, contact between donor and recipient is unstable at first but with time it is stabilized by an unknown mechanism.

Noted also was the fact that the transfer of ampicillin and tetracycline resistances accurred both separately or in combination regardless of whether the isolates were from Birmingham or Dar es Salaam. This suggests three separate plasmids, one for tetracycline the second for ampicillin and the last for the two antibiotics. It could be argued also that the different isolates showing resistance to tetracycline and ampicillin haboured a common plasmid. Characterization of the latter plasmid perhaps could have revealed more information.

Although kanamycin resistance occurred very rarely, transfer of kanamycin resistance occurred with streptomycin resistance, again, suggesting a common plasmid.

Chloramphenicol resistance is also plasmid mediated. A plasmid specified drug permiability barrier has been suggested as the mechanism of chloramphenicol resistance determined by some R-factors that do not encode chloramphenicol acetyl transferase (CAT) (Foster et al 1981).

Nalidixic acid and nitrofurantoin resistance were not transferable on any occasions. However, they have both been shown to be plasmid mediated, though for nalidixic acid, it has not been fully substantiated Labek G. (1963).

Due to the fact that all organisms isolated in this study were sensitive to gentamycin, transfer experiments were not possible but it has been shown by various workers to be plasmid mediated and transferable by conjugation (Mitsuhashi 1982; MacDonnel et al 1983; Smith and Lus 1975).

Sulphonamide resistance has also been shown to be plasmid mediated (Davey and Pittard 1974).

Another important observation made in this study was that although there were more Escherichia isolates from Birmingham samples than in Dar es Salaam, the percentage that transferred resistance in the former was lower than expected. The high percentage of Escherichia isolates from Birmingham samples was thought to reflect a high mating compatibility between donor and recipient, E.coli K12 but this was not the case. It is suggested therefore that among other reasons there was a possibility for loss of the R-factor during storage as they had been kept for quite a considerable period of time.

Additional reasons why transference did not occur in all other isolates include:

1. The resistance of donors might have been chromosomal in origin.

2. When DNA is transferred to a new host, and when this recipient is not genetically identical to the donor, particulary when it comes from a different species, the transferred DNA is frequently non-functional. In some instances it is degraded by endonucleases of the recipient; however in at least one instance, it survives and is transcribed but not translated.

3. As regards Klebsiella isolates, most of them carry a strong (ML+) or a weak (ML-/+) lactose positive phenotype on MacConkeys agar. Most strains also carry a lac operon plasmid which can be transferred to E.coli. It is suggested that transcipients could have easily been mistaken for donors if the latter transferred the lac operon to E.coli K12S and only one antibiotic was used for selection (Reeves and Brainthwaite 1973).

4. E.coli K12S and E.coli K12N may have not been universal recipients.

5. Operator errors.

6. It is possible that some strains carry R-determinants but have lost the ability to conjugate and transmit resistance (loss of RTF).

7. Many bacterial species habour plasmids which are termed 'cryptic' because they confer no obvious phenotype on their host cells. Plasmids of this type constitute a majority in some bacterial groups (Croft et al 1983).

8. In case the chromosomal resistance was also transferable by conjugation, it suggests a delay in expression of the mutation (segregation lag). It also might be due to a phenotypic-lag. This is because many rod shaped bacteria including the most throughly studied species E.coli and S.typhimurium tend to have two or more chromosomes per rod. Each rod in reality, is equivalent to two or four cells, with one chromosome in each, though the convinient practice of referring to the whole rod as a cell is very general.

Isolates from Dar es Salaam samples were not tested for cotrimoxazole and trimethoprim sensitivity. This was because the neosensitabs were spoilt during storage. Attempts to get fresh ones were unsuccessiful. This means, some of the isolates which were sensitive to all drugs tested might infact have been resistant to these drugs.

With regard to transfer between unknown donors and unknown recipients, it was possible to get 1 transfer out of 59 samples. In the real sense this represents a serious problem because in a population of 20 million this would be very significant.

## 4.3 IMPLICATION OF TRANSFERABLE DRUG RESISTANCE FACTORS IN THE INCREASE IN RESISTANCE TO DRUGS USED IN THE TREATMENT OF U.T.I. AT M.M.C.

Retrospective studies of urinary tract infections at Muhimbili Medical Centre as from 1970 to 1983 have revealed that microbial resistance to the various antimicrobial drugs is on the increase. As mentioned earlier in the text, the increase is not uniform but with peaks and valleys. (Graph 1 pg. 40).

The organisms frequently isolated in U.T.I. at Muhimbili Hospital include E.coli, Klebsiella-Enterobacter species, pseudomonads and Staphylococcus aureus. The percentage of these organisms showing resistance to tetracycline, ampicillin and sulphonamides is extremely high. By 1980 for example, pseudomonads (Graph 1) had become completely resistant to tetracycline. If the latter fact is extropolated and taking into account the rate of development of resistance, it may not be very long before E.coli Klebsiella species and other organisms become completely resistant to not only tetracycline, ampicillin and sulphonamides but also to other antimicrobial drugs as well. Because E.coli is so overwhelmingly important in the development of urinary tract infection, there has been interest in the sources of strains of E.coli causing infection and in the nature of the infecting organisms. There are two current views about this. The 'prevalence theory' is that the organisms causing the infection are merely those commonest in the faeces of the population under consideration. The 'special pathogenicity' theory is that organisms causing the infection are a selected group which have properties which specially allow them to invade or multiply in the urinary tract. A number of properties have been considered to be associated with pathogenicity in E.coli; these include serotype; haemolysin and necrotoxin production, cytotoxicity, the K antigen, and the presence of fimbriae (Montgomerie 1980).

In recent years, evidence has accumulated for various bacterial infections, which show that the adherence of bacteria to mucosal surfaces of susceptible hosts is an important step in colonisation and infection (Ofek and Beachey 1980). The ability of E.coli to adhere to epithelial cells is often associated with fimbriae which can be classified into various types by dimensions, composition, stability, antigenicity, adhesiveness, specificity and agglutination patterns with erythrocytes and yeast cells (Duquid and Old 1980).

Fader et al (1979) found that the fimbriae of Klebsiella pneumonia were similar to type I fimbriae of E.coli and play an integral role in the ability to cause cystitis in rats by mediating the attachment of the bacteria to the uroepithelial surface.

The results of the present study and the restrospective studies by Douglas et al (unpublished observations) and Maselle et al (1980) agree very well with the 'prevalence theory'. It is also very likely that the 'special pathogenicity' theory may hold.

The list of organisms causing U.T.I. at M.M.C. also include pseudomonads and Staphylococcus aureus which were not delt with during the present study. However, transferable drug resistance in Pseudomonas species is common knowledge. Bryan et al (1972) demonstrated the transfer of double drug resistance between Pseudomonas aeruginosa species and also to E.coli by conjugation.

In Staphylococcus aureus, transferable drug resistance has also been shown by various workers (MacDonell et al 1983; Cohen et al 1982; Schaberg 1982). Furthermore it was shown that transformation, transduction and conjugation were all potential mechanisms for genetic exchange (Forbes and Schaberg 1983).

Maselle and others (1980) reported that the microbial resistance to commonly used antimicrobial drugs at M.M.C. was proportional to the amounts of drugs used. However, the reverse is also true, that is, examples may be cited where resistance varies inversely to the amounts of drugs used (Table IVa).

Since the statements above are both true, then by analogy, the former cannot explain why in 1983 the percentage of E.coli resistant to sulphonamides fell from (92%) to (29%) at the expense of consuming (893Kg) of the drug (Table IVb).

While it is appreciated that resistance is more often falsely reported than sensitivity, the theory that drug resistance is associated with the extensive use of antibiotics does not explain the origin of resistance in all cases. The latter, however, may explain the sulphonamide case, only if, the resistance is plasmid mediated.

The theory of spontaneous gene mutation maintains that resistance to drugs may occur even in the absence of antibiotics. However, mutation rates are of the order 1 in $10^7$, therefore, too low to account for the rapid spread of bacterial resistance to drugs used in the treatment of U.T.I. at M.M.C. viewed from a theoretical point if $10^{-a}$ and $10^{-b}$ show mutation frequence of resistance to A and B respectively, then $10^{-(a+b)}$ will be equal to the simultaneous resistance to both drugs, development of one being independent of the other. This indicates the infinitesmal chance of simultaneously developing both mutation in a single cell. (Mitsuhashi, 1975).

Plasmids, have been cited to be very important in drug resistance. An important aspect of the acquisition of plasmids by pathogens is that the entire resistance spectrum, extending to as many as seven drugs, may be acquired in one or very few events (W.H.O. 1976).

In this study isolates from healthy human subjects containing R-factors to various antimicrobial drugs transferred resistance to E.coli $K_{12}$. Although with difficulty, it was also possible to transfer R-factors from "Unknown" donors to "Unknown" recipients implying that the process can proceed spontaneously in the individuals own bowel. Furthermore, E.coli isolates from patients with U.T.I. at M.M.C. were able to transfer resistance to E.coli K12S.

Montgomerie (1980) reported that the majority of U.T.I. especially of acute and uncomplicated episodes are caused by E.coli. He further added that the prevalence of E.coli in such infections is assumed to result in its proximity to the G.I.T. This agrees well with the M.M.C. case because the organisms isolated from stools of healthy human subjects are highest on the list of U.T.I.

To emphasize the importance of the above statements, a good number of Dar es Salaam residents have a habit of using [illegible] water to wipe their anal areas instead of toilet tissue as dictated by example religion or sometimes shortages. The author is of the opinion that the above fact may have played an important role in the dissemination of R-factors, especially in U.T.I.

The practice of extranatural sexual practices, eg sodomy perhaps poses yet another factor which may favour the spread of the R-factor. A study by Dudek et al (1982) showed that this is true. He collected (43) isolates of Neisseria meningitidis from known male homosexuals. Of these (21) were from the oropharynx, (15) were from the rectum and (7) from the urethra. This shows how microorganisms get to places where they do not belong.

At this point, it is possible to suggest that the increase in microbial drug resistance to drugs used in the treatment of U.T.I. at M.M.C. may be R-factor mediated.

The author is of the opinion that the peaks in the graph (Graph 1) suggest additionally acquired R-determinants while the valleys indicate deletion variants of unstable plasmids. For example a plasmid with resistance determinants to seven drugs, may become unstable and loose some determinants so as to become stable. The latter example can be used to explain the sulphonamide case. The plasmid, perhaps becomes too long to be accommodated on the same dimensions and is chopped off a bit at one or both ends. Chau et al (1982) observed the resistance of Salmonella johannesburg to six drugs Amp, Str, Tet, Chl, Kan and Sud for 7 years from 1973 to 1979. Strains with Amp, Str, Tet, Chl, Kan, Sud and Amp, Str, Chl, Kan, Sud - resistance patterns predominated in the years 1973-1975 and 1976-1979, respectively. These resistances were found to be mediated by autotransferring plasmids belonging to the incompatibility group FIme. The Amp, Str, Tet, Chl, Kan, Sud - resistance plasmids were unstable, giving rise to deletion variants at a much higher frequency than Amp Str Kan Sud resistance plasmids either of natural origin or derived in vitro from Amp, Str, Tet, Chl, Kan, Sud resistance plasmids. Thus it was suggested that the Amp Str Chl Kan Sud-resistance plasmid might be a deletion variant of the Amp, Str, Tet, Chl, Kan, Sud resistance plasmid.

In summary, drug resistance is of abvious ecological advantage to bacteria in an environment where antibiotics are extensively used. This explains the prevalence of antibiotic resistant coliforms in healthy human subjects and in hospitals.

Sud = sulphadiazine.

The presence of plasmids play an important role in bacterial resistance in addition to the mutation based on bacterial chromosomes. As Mitsuhashi (1975) put it "Genetics proposes, Nature disposes".

The problem of antibiotic resistance is a real one and cannot be solved by introduction of new drugs. It is not surprising at all to find a number of bacteria in a population which are resistant to antibiotics which have not yet been introduced.

## 4.4. CONCLUSION AND RECOMMENDATIONS:

The present study has shown that R-factors are somehow responsible for the drug resistance found in Gram-negative bacteria of healthy human subjects in Birmingham and Dar es Salaam. Some of these resistances may be transferable to E.coli K12. From transfer experiments, it appears that the resistance is, at least in some cases, R-factor mediated.

Furthermore it appears from retrospective antibiotic use and resistance studies that the high level of microbial resistance to drugs used in the treatment of U.T.I. at Muhimbili Medical Centre may be plasmid-mediated.

The author suggests immunological approaches to the problems of infections causes by antibiotic resistant organisms.

Mass education on the problems of resistance and indiscriminate use of antibiotics may help to arrest the increase in microbial resistance.

It seems that exclusive use of toilet tissue (as discussed earlier) might in some communities, help to decrease R-factor mediated resistance to antibiotics. However, if reasons such as cultural, personal or otherwise arise in favour of water, then alternative ways should be sought. One way is to have specially designed toilets i.e. the pedle toilet (Appendix V). Extra-natural sexual practices should be discouraged through sex-education classes. However, there is room for research to compare the above from the rest of the population.

With the present rate of appearance of drug resistant organisms in Dar es Salaam, an antibiotic policy is mandatory if the values of antibiotics have to be maximized.

It is recommended therefore that, antibiotics be divided into three groups as follows:-

(i) First-line This group should include drugs which are cheap, effective and with a low percentage of resistance. Examples would include nalidixic acid and nitrofurantoin (Table VIII).

(ii) Second-line This group should be controlled. Drugs in this group should not be dispensed otherwise than in accordance with a culture and sensitivity-test report signed by a qualified medical microbiologist. The report should also indicate that other antibiotics have failed. Gentamycin, kanamycin and cloxacillin should fall in this group.

(iii) Restricted-Drugs: This group should include the drugs to which organisms have shown high resistance. These include tetracycline, ampicillin, sulphonamides, chloramphenicol and streptomycin. The group should be reserved only for special cases where the drugs are specifically indicated. In this way the resistant determinants may get lost.

Lowbury et al (1975) have summarised Hospital antibiotic policies for the years 1971 and 1974. They have classified the antibiotics into three categories, depending on: a) availability of antibiotics

b) reasons for choice and

c) the antibiotics to use.

In some cases, similarities to the proposed Muhimbili Medical Centre antibiotic policy do occur. However, differences are also very obvious.

This is because unlike Tanzania, developed countries have a wider selection of antibiotics. The 1971 antibiotic policy (Dudley Road Hospital) for example, categorises tetracycline in category 1, i.e. with no special restrictions in treatment of sensitive infections when clinically required. However, it is also covered in category 3, that is, restricted antibiotics so as to restore activity when most strains are resistant. This kind of classification (i.e. the 1971) would also be acceptable for M.M.C. except for urinary tract infections, where tetracycline would not fall in category 1. This is because resistance to this antibiotic is very high. The 1974 antibiotic would be much more appropriate for M.M.C. but drugs such as cloxacillin, flucloxacillin, ampicillin, amoxycillin and cotrimoxazole are too expensive to be included in category 1 in the case of Tanzania.

Another alternative would be the rotational use of antibiotics. The method has been used by a number of countries. It is however, a very expensive approach for developing countries but if need be, then Tanzania will have no choice.

No antibiotics of therapeutic value in human or showing cross resistance with such antibiotics should be used to promote growth in animals. However, this policy will not work unless the use of such antibiotics to prevent and treat diseases in animals is also restricted.

The therapeutic use in animals of newer antibiotics used to treat serious infections in man, should be prohibited.

Unless steps are taken to check the misuse of antibiotics which lead to resistance, one of the best weapons humanity has devised for protection and restroration of health could be in jeopardy.