INTRODUCTION & AIM OF WORK

INTRODUCTION AND AIM OF WORK

Ps.aeruginosa is a world wide distributed and multiplies freely in the inanimate environment .It causes a wide variety of specific infections in man and other vertebrates such as; infection of skin & wounds, abscesses in the internal organs and may even give rise to acute septicemic infections (Topley and Wilson's 1984). Infections caused by *Ps.aeruginosa* have become increasingly prevalent in the hospitals. Moreover, chemotherapy of these infections presents a problem since *Ps. aeruginosa* is naturally resistant to most of the commonly employed antimicrobial agents (Lowbury, 1975).

It is a common phenomenon in most general hospitals that the frequency of occurrence of infections due to *Ps. aeruginosa* is increasing. The reasons for the increase are not completely understood, but undoubtedly they bear some relationship to the wide spread use of antibiotic therapy and the resistance of *Ps.aeruginosa* to the most of the widely used antibiotics (Holloway, 1969). It is not unusual for strains of this organism isolated from infections to be resistant to three or more of the following antibiotics, sulpha-

diazine, ampicillin, kanamycin, streptomycin, chloramphenicol, neomycin or tetracycline. The only antibiotic to which *Ps.aeruginosa* is usually sensitive is polymyxin, although strains resistant to this agent are common (Holloway *et al.*,1979).

Smith et al.(1975) showed that the drug resistance of *Ps.aeruginosa* could be transferred to sensitive *E. coli.* cells. Similarly Lebek (1977) showed that an *E. coli* strain could transfer its RTF to drug sensitive *Ps.aeruginosa* strains.*Ps. aeruginosa* has been reported to be resistant to a wide range of antibiotics (Berzeziska et al., 1972). Moody *et al.* (1970), tested 332 strains of *Ps. aeruginosa* for their antibiotic resistance and he reported that kanamycin resistance was 90%. Ashour(1980) , revealed that among fifty-two isolates tested , 88.4 % were resistant to kanamycin, 4% to carbenicillin and 2% to gentamicin and polymyxin. Zowel(1982) showed that resistant percentage was 78.51% among seventy isolates tested; 88.57% ,77.15% ,25.71%, 31.45% and 8% were resistant to kanamycin , carbenicillin ,tobramycin, gentamicin and amikacin ,respectively . El-Naggar(1984) reported that among 250 isolates collected, 48.6% of the isolates were resistant to carbenicillin , 42.4% were resistant to kanamy-

cin ,6% gentamicin resistant , and 2.6% were amikacin resistant On the other hand Mokhtar (1985) found that 90% of the *Ps. aeruginosa* isolates were resistant to carbenicillin and 50% to gentamicin while only 8% of the isolates were tobramycin resistant and non resisted amikacin. Ashour *et al.* (1987) revealed that among seventy tested isolates, 87.14% were resistant to amoxycillin ,74.28% to nalidixic acid ,67.14% to kanamycin, 25.71% to tobramycin, 21.42% to carbenicillin, 11.4% to gentamicin and 7.14% to amikacin.

Resistance of some *Ps. aeruginosa* strains to tobramycin and gentamicin was attributed to R plasmid. However some of these R.plasmids may determine resistance to gentamicin but not to tobramycin (Jocoby, 1974 a & b). On the other hand Bryan *et al.* (1984) concluded that low level impermeability type aminoglycoside resistance in *Ps. aeruginosa* resulted from the conversion of smooth Lipopolysaccharide (LPS) to superficial or deeper rough LPS phenotype.

Lowbury (1975) mentioned that gentamicin and tobramycin are usually active enough to have potential value in therapy of *Ps. aeruginosa* infections. Also Dulong De Rosnay *et al.* (1976) reported the activity of both tobramycin and gentamicin, while Duval and Soussy

(1980), reported the activity of amikacin in addition to tobramycin and gentamicin. From Egypt, Ashour (1980)revealed that the most effective antibiotics were gentamicin, polymyxin and carbenicillin, while Abd-Elatif (1982) showed that the most effective antibiotics were carbenicillin, gentamicin and amikacin. Rabie(1984) showed that among 24 isolates collected from burns the most effective antibiotics were; gentamicin, carbenicillin, kanamycin and nalidixic acid. The same investigator (1984) also reported the results of sensitivity of 20 urinary tract isolates. The gentamicin was the highest active antibiotic followed by carbenicillin and nalidixic acid while kanamycin was totally ineffective.

Variation of susceptibility of *Ps.aeruginosa* to gentamicin and tobramycin could be explained through the fact observed by several authors that MIC of these antibiotics increases when the concentration of calcium and magnesium in the test medium are raised (D 'Amato *et al* .,1975).

Antimicrobial chemotherapy has played an extremely important role in the fight to overcome the bacterial pathogen that produce infectious diseases. In 1877, Pasteur and Jubert were the first investigators to

recognize the clinical potentialities of microorganisms as therapeutic agents, they noted that Anthrax bacilli grew rapidly when inoculated into sterile urine but failed to multiply and soon died if one of the common bacteria of air was introduced in the urine at the same type (Gilman *et al.*, 1971).

The word antibiosis was coined by Vuilleman in 1889 to denote antagonism between living creatures in general (Garrod and O'Grady, 1971).

Paul Ehrlich 1906 stated that in order to use chemotherapy successfully, it must search for substances which have an affinity for the cells of parasites and the power of killing them greater than the damage such substance causes to the organs itself (Laurence, 1970).

Rapid development in antimicrobial chemotherapy began in 1935 with the discovery of sulphonamides by Domagk. In 1940, Chain and Florey demonstrated that penicillin, which had been observed in 1929 by Fleming, could be made an effective chemotherapeutic substance. During the next 25 years, the chemotherapeutic research largely centered around antimicrobial substances of microbial origin which were called antibiotics. Antibiotics include the culture extract and filtrate of

fungi, bacteria and bacillus species. In recent years, chemical modification of molecules by biosynthesis has been a prominent method of new drug development (Topley & Wilson's,1984).

The chemotherapeutic agent "chemical antimicrobials"is a synthetically produced antimicrobial compound (Cheesbrough, 1985).

During the last 15 years, outbreaks of disease due to multiresistant strains of enteric bacterial pathogens have occurred. The outbreaks have involved most of the important etiologic agents of bacterial diarrhea. The resistance patterns exhibited by these organisms have included those antibiotics that were being used most heavily at the time of outbreak, as well as older agents and the resistance markers were usually encoded on one or more plasmids (Edmund, 1986).

Resistance to antibacterial agents has been a major concern. Until now every drug introduction has been followed by the emergence of resistant strains. There are only few exceptions to this rule, individual species, such as the unique susceptibility of *Streptococcus Pyogenes* to penicillin or *Staphylococcus aureus* to vancomycin. (McGowan,1983).

Plasmids are extrachromosomal DNA elements of bacteria. Resistant plasmids (R-factors) are a class of plasmids that carry genes for resistance to one or several antibiotics. Plasmids have been found in a wide variety of bacteria including *Pseudomonas* as mentioned previously. (Michael and Greco, 1982).

It is now clear that many of the pathogenic properties of the *Clostridium tetani, Anthrax bacilli* and *Yersinia species* are plasmid mediated (Larid *et al.*, 1980, Portnoy *et al.*, 1981, Mikesell et al., 1983 and Portnoy et al., 1983). Moreover, the invasive properties of *Shigella* and enteroinvasive *E. coli* are dependent upon specific plasmid (Hale *et al.*, 1983). Also the enterotoxin productivity of enteropathogenic *E.coli* strains is encoded by plasmids (Kleckner, 1981).

The aim of the present work is to clarify the genetic basis of antibiotics resistance displayed by *Ps.aeruginosa*. To reach this point some isolates of *Ps. aeruginosa* highly resistant to some antibiotics will be isolated. The type of antibiotic resistance whether it is chromosomal or extrachromosomal (plasmid) will be determined. Probably through plasmid isolation and *E. coli*, K12 transformation one can emphasize whether plasmids are responsible for these resistance or they

are due to other genetic factors. The role of phage in antibiotics resistance will also be determined. Attempts for plasmid curing through various methods will also be carried out.