INTRODUCTION

The emergence of Pseudomonas aeruginosa in the last two decades as an important opportunistic pathogen in nosocomial infection can be attributed to factors relating to both the organism and human host. First, Pseudomonas aeruginosa has a wide spread distribution in nature. Physiological adaptability and innate resistance to many antimicrobial agents. Second, there is an increasing number of patients who are susceptible to infection with such organism because of age, debilitation, treatment with antibiotics, immunosuppressive agents or antimetabolities. The range of Pseudomonas infection is extensive and virtually no part of the human body is sacrosanct.

These factors and the difficulty encountred in successfully treating established infections make it essential constantly to monitor the different strains of Pseudomonas aeruginosa present in the hospital environment in order to determine the mode of spread of a particular strain in an outbreak (Clarke & Richmond, 1975).

Three methods have been used to type Pseudomonas

aeruginosa, seretyping, phage typing, and pyocin typing. (Gillies & Govan 1966, Govan & Gillies 1969 and Govan, 1978).

Aim of work:

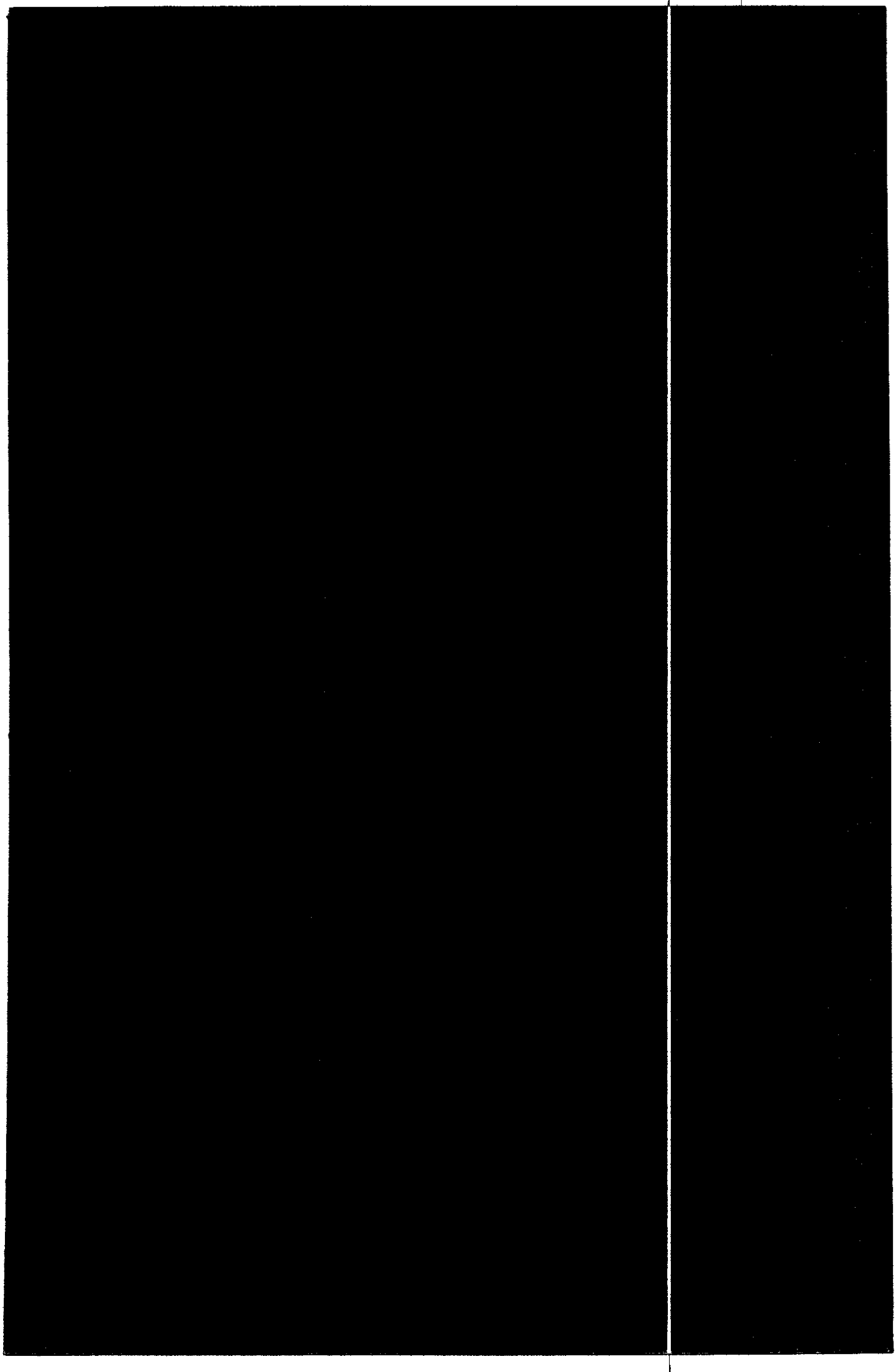
1- Isolation of some strains of Pseudomonas aeruginosa from burns, wounds and urinary infections in the hospitals of Zifta and Benha.

2- Identification of the previous strains by biochemical reactions and Gram Stain.

3- Determination of antibiotics sensitivity patterns of the isolated strains by the disc agar diffusion method using the following antibiotic discs, amikacin, gentamycin, tobramycin, polymyxin - B, colimycin, claforane and carbenicillin.

4- Determination of pyocin types of the isolated strains of Pseudomonas aeruginosa using the indicator strains of Govan pyocin typing set.

5- The use of some selected Pseudomonas aeruginosa strains



REVIEW OF LITERATURE

Ps. aeruginosa is the causative agent in bacteraemia leading to the highest mortality rate (Bryan et al.,1983). Pseudomonas was first described by Schroeter (1872) under the name Bacterium aeruginosa:

Bacillus Pyocyaneus	(Gessard 1882)
Micrococcus Pyocyaneus	(Zopf 1884)
Bacillus aeruginosa	(Trevisan 1885)
Ps. Pyocyanea	(Migula 1895)
and Ps. aeruginosa	(Migula 1900)

Isolation and Identification:

Ps. aeruginosa, the primier pathogen of Pseudomonas species, is a leading cause of mortality in compromised hosts. Other species frequently encountered in the environment are genetically, biochemically and physiologically similar to Ps. aeruginosa but none shares its pathogenic potentialities(Vasil 1986). Ps. aeruginosa is a gram negative straight or curved rods motile by means of one or more polar flagella, some strains also have

lateral flagella of different lengths and one species is non motile, non sporing and not acid fast, strict aerobes but some grow anaerobically in the presence of nitrate (Toply and Wilson 1984).

Ps. aeruginosa grows readily on culture media does not ferment lactose and forms smooth round colonies with fluorescent greenish colour and a sweetish aromatic odour. From the colonies bluish green pigment diffuses into the medium among pigments produced by Ps. aeruginosa is pyocyanin, bluish material soluble in chloroform and water (Jawetz et al., 1984).

When Ps. aeruginosa grows in nutrient broth it shows uniform turbidity and surface pellicle. Colonies formed by streaking of Ps. aeruginosa. On nutrient agar are large low convex rough and often oval. Pale colonies are obtained on MacConkey's agar i,e lactose non fermenter (Cruickshank et al., 1975). Using blood agar similar growth on nutrient agar but the pigments are not well demonstrated (Wilson and Miles 1964). Haemolysis may be

detected as a result of production of soluble haemolysin or lecithinase enzyme (Liu et al., 1961). Ps. aeruginosa can grow at higher temperature 43°C than the enteric organism and can utilize over 50 simple organic compounds for growth (Vasil, 1986) and it will grow in an anaerobic environment if nitrates are available. Ps. aeruginosa attacks glucose oxidatively with acid. Fourty percent of Ps. aeruginosa strains examined by Colwell (1964) produced acid from galactose. No acid is formed from lactose, maltose, mannital sorbitol, dextrin and glycerol. Starch is not hydrolysed, cellulose and pectin are not attacked. Voges proskaur and methyl red reaction are negative, methylene blue is not reduced (Wilson and Miles 1964).

Litmus milk is decolourised completely in 3 days at 30°C, there may be slight preliminary clot (Wilson and Miles 1964). Casein is hydrolysed (Colwell 1964). Ammonia formed from arginine, gelatin is rapidly liquified, H_2S is not produced, indol negative (Wilson and Miles, 1964) oxidase positive (Gordon and MacLoad 1928 and Kovacs, 1956). It deaminate acetamide (Topley and Wilson, 1975).

Ps. aeruginosa distinguished also from other Pseudomonas in its production of pyocyanin, blue phenazine pigment that gives the colour blue pus. This pigment and several others such as pyochelin and pyoverdin are secondary metabolities of Ps. aeruginosa and are thought to play an important role in its nutrition (Vasil, 1986). Pyocyanin may play a role in acquiring inorganic phosphate and compates with other microorganism by means of its antibiotic activity. It may also be critical in iron metabolism by increasing the solubility of iron accumulated through production of pyochelin and pyoverdin which may be necessary for the aquisition of iron by Ps. aeruginosa in an infected host (Cox, 1985). King et al.,1954 stated that under artificial conditions of laboratory cultivation many strains of Ps. aeruginosa tend to loss their ability to produce pyocyanin. Utilization of nitrates could explain the ability of Ps. aeruginosa to survive in environments such as soil and more important certain hospital environment. The production of protease and the anaerobic exploitation of their end. Products

particularly arginine may enable Ps. aeruginosa to initiate infection in parts of the body where little or no molecular oxygen is available (Vasil, 1986).

Table (1): Characteristics of major known virulence factors of Pseudomonas aeruginosa (Vasil,1986)

	Composition/mechanism of action	Genetics	Potential role	Reference
- Exotoxin A	66,500 mol wt peptide inhibits ADP ribosyl transferase	Chromosomal gene in 95% of strains	Invasivness lethality Immune suppression	(Vasil et al.,1985-Vasil & Iglewski, 1978 - Ogle & Vasil, 1985-Woods et al., 1982).
- Haemolysin Phospholipase C	78,000 mol wt peptide-degeneration of elastin.	Chromosomal gene in 100% of strains.	Invasivness Scavening of nutrients (Pi).	(Vasil et al.,1985-Southern et al.,1970-Pallaroni etal.,1984)
- Elastase	39,000 mol wt peptide degeneration of elastin.	Chromosomal gene highly variable expression	Invasivness,local necrosis,destruction of IgG IgA, C_3b, C_3a	Woods et al.,1982-Granstrem et al.,1984-Wretlind & Pavlovskis 1981-Doring et al., 1983).
- Exotoxin S	Variable mol wt polypeptide(s) inhibits ADP ribosyl transferase	chromosomal gene? 40% to 80% of strain produce toxin.	Dissemination, local tissue destruction	(Nicas et al.,1985 - Iglewski et al.,1978-Woods & Sokol,1985).
- Cytotoxin (Leucocidin)	25,000 - 42, 500 mol wt protein	?	Kills leukocytes particularly polymorpho nuclear leukocytes.	(Pier, 1985).
- Pili	Polypeptide with 15,000 mol wt subunit	chromosomal gene	specific adherance.	(Pier, 1985).
- Alginate Mucopolysaccharide.	Polymer of guluronic and mannuronic acid	Several chromosomal genes required for synthesis	Inhibition of phagocytosis adherance Immune damage	(Reynolds, 1985 - Ohman & Chakrabarty 1982 - Stapleton et al., 1984).

The observation that Ps. aeruginosa endotoxin was minimaly toxic compared with other gram negative endotoxin, but the lethality of Ps. aeruginosa infections was relatively high, led to search for the discovery of an additional toxic factor, exotoxin A, the single most toxic substance on the basis of weight, produced by Ps. aeruginosa (Iglewski & Kabat 1975). Subsequent studies suggested that the exotoxin A acted on protein synthesis (Iglewski & Kabat 1975). The most significant finding on the biochemistry of exotoxin A was presented elegantly by Iglewski and Kabat in 1975 who reported that exotoxin A catalyze the transfer of the adenosine disphosphate ribosyl-moiety of NAD onto eucaryotic elongation factor 2, which is critical to protein synthesis. This enzyme actively is identical to that of diphtheria toxin. Further study of the biochemistry structure function relationship and genetics of exotoxin A has revealed interesting parallels between diphtheria toxin and exotoxin A, and some even more interesting contrasts. Exotoxin A and diphtheria toxin conform to the A - B structure function model of many bacterial toxins. The B fragment of the molecule

is necessary for interaction with eucaryotic cell receptor. The A fragment is catalytic. An important difference between exotoxin A and diphtheria toxin which could account for the vast clinical diversity in the diseases they cause, is that the specificities of these toxin for different kinds of eucaryotic cells are distinct. The most probable explanation is that the eucaryotic cell receptors for these toxins are not the same (Vasil and Iglewski 1978). The production of both toxins is regulated by environmental iron concenteration (Vasil et al., 1985). When iron is limited exotoxin A is optimally produced. If iron levels are sufficient for optimal growth of the organisms, the synthesis of toxin is repressed (Vasil et al., 1985). The production of diphtheria toxin depends on a bacteriophage designated (beta). This phage and other corynebacterium phages carry the structural gene for diphtheria toxin. In contrast, the structural gene for exotoxin A appears to reside on the chromosome in at least one strain (Howe et al., 1983 and Vasil et al., 1985). The possibility of a similar relationship between exotoxin A production and a bacteriophage

has not been rigorously excluded but none has been found. The exotoxin A gene has been cloned. Radiolabeled probes derived from the exotoxin A structural gene have been used to study the epidemiology of the exotoxin gene in Pseudomonas (Vasil et al., 1985 - Ogle and Vasil, 1985). It was found that only Ps. aeruginosa strains carry sequences capable of encoding exotoxin A and there is only one copy of the exotoxin A gene per cell, therefore gene amplification is not a mechanism explaining the highly variable production of exotoxin A by different strains of Ps. aeruginosa. Four strains have been found to lack exotoxin A structural gene and three of these four strains were isolated from patients with cystic fibrosis. Colonial variants of Ps. aeruginosa from a single patient with cystic fibrosis different considerably in their ability to produce exotoxin A as well as phospholipase C (heat labile haemolysin) and elastase. It is unlikely that this patients was colonized by multiple Ps. aeruginosa strains. Some colonial variants were of different serotype and biotype but had identical restriction

endomuclease patterns (Ogle & Vasil, 1985). They reacted in an identical manner with a DNA probe that has more discriminating power than serotyping and biotyping combined (Ogle and Vasil, 1985).

In the chronically infected rat lung, the experimental animal model that most closely mimics Ps. aeruginosa infection in cystic fibrosis, production of exotoxin A appears to contribute to disease, but it is not essential for virulence (Woods et al., 1982).

Klinger et al.,(1978) and Jagger et al.,(1982) demonstrated an inverse correlation between antiexotoxin A antibody titre and clinical scores in patients with cystic fibrosis. On the basis of these data. It seems probable that a protein as toxic as exotoxin A produced in vivo during an infection, contribute to the pathogenesis of that infection.

The phospholipase C or heat labile hemolysin of Ps. aeruginosa has not been throughly studied in exotoxin A. This enzyme hydrolyses certain phospholipids into diacyl

glyceral and phosphoryl choline. Phospholipase C has a limited substrate specificity, perferentially acting on phospholipids containing quaternary ammonium groups, for example, phosphatidylcholine, lysophosphatidyl choline and sphingomyelin (Vasil et al., 1985). These phospholipids are abundant in eucaryotic cell membrane but scarce in procaryotic membrane. Phosphatidylcholine is the major phospholipids component (75%) of lung surfactant. This surfactant is critical to lung physiology and may function as a nonimmune opsonic factor for alveolar macrophages (Reynolds 1985). Based on the enzymatic specificity of phospholipase C, production of phospholipase C could contribute to the virulence of Ps. aeruginosa in the lung. Preliminary studies support this hypothesis. Bronchial washing alone will support the production of phospholipase by Ps. aeruginosa suggesting that this enzyme can be produced in vivo (Liu, 1979). Production of phospholipase C is regulated by inorganic phosphate: as environmental Pi concentration decreases, phospholipase C production increases. These observations suggest that this potential virulence factor is produced in vivo.

In a recent study, 100% of patients with cystic fibrosis infected with Ps. aeruginosa produced antibody against phospholipase C (Granstrom et al., 1984). The titre of antiphospholipase antibodies correlated with chronic colonization rather than intermittent colonization. Southern et al., (1970) found that clinical strains of Ps. aeruginosa producing the highest amounts of phospholipase were poorly cleared from the lung and actually able to multiply. In contrast strains producing relatively low amounts of phospholipase were cleared more rapidly and not observed to multiply in the lung. It has been hypothesized that, together with alkaline phosphatase and other Pi binding proteins, phospholipase C may be critical for Ps. aeruginosa to "scavenge" Pi from phospholipids or other organic compounds. Infection, with tissue destruction, would then release Pi. Ps. aeruginosa has an extra ordinarily high requirement for Pi, probably because of the high Pi content of its outer membrane. Pi may bind cations and link the lipopolysacchoride to other wall components. (Palleroni, 1984). Isolates from infected lungs of patients with and without cystic

fibrosis are the most variable in their ability to produce phospholipase C. More recently, using the cloned phospholipase gene as a probe, it was found that 100% of more than 80% Ps. aeruginosa isolates examined carry the phospholipase gene. Examination of numerous Pseudomonads other than Ps. aeruginosa indicates that they do not have the gene for phospholipase C.

Proteases have a limited role in the pathogenesis for Ps. aeruginosa infection. Their action is restricted to the focus of infection because their activities are inhibited by the serum α 2 - macroglobulins. Wretlind and Pavlovskis (1981) appropriately described elastase, one of the more specific proteases as " a virulent - enhancing factor in certain types of infections". Virulence factors like proteases are also referred to as "aggressins". In addition to causal local tissue destruction, aggressins may also enhance virulence. Recent studies suggest that elastase and an alkaline protease inhibit neutrophil chemotoxis in vitro. Elastase may inactivate C_3 b and C_5a

complement components and there by inhibit opsonization and generation of chemotactic factors. It is conceivable that this property promotes invasiveness of Ps. aeruginosa. Proteases have also been shown to have activity against IgA (Doring et al., 1983).

Doring et al., (1983) have demonstrated that patients produce antibodies to elastase and an alkaline protease. These enzymes were detected in bronchial secretions when specific antibodies to them were not present in sera and bronchial secretion, suggesting that protease are neutralized by production of specific antibodies. Ohman and Chakrabarty (1982) recently observed that mucoid and non mucoid isogenic variants of Ps. aeruginosa strains from patients with cystic fibrosis varied significantly in their ability to produce elastase. The non mucoid variant produced greater amounts than did the corresponding mucoid variant.

One of the more recently discovered virulence factors of Ps. aeruginosa is exoenzyme S, also called exotoxin S (Iglewski et al., 1978). It is particularly

interesting because it has adenosine diphosphate ribosyl transferase activity similar to but distinct from that of exotoxin A. There is no immunologic cross reactivity between these molecules, and the substrate for exotoxin S has not been clearly delineated. Because exotoxin S is recently discovered and has not been purified to homogeneity, little is known about the role of this extracellular protein. Mutants deficient in their ability to produce exotoxin S are less pathogenic in the chronic rat lung model, or exotoxin S may be not required for maintenance of the chronic infection (Nicas et al., 1985 - Woods and Sokol, 1985). In some studies not even a simple majority of cystic fibrosis Ps. aeruginosa isolates produce exotoxin S. One estimate based on the most sensitive method is that only 43% of strains from patients with cystic fibrosis produce detectable exotoxin S (Nicas et al.,1985), but these may be the most virulent strains.

Perhaps the most important virulence factor in Ps. aeruginosa infection in patients with cystic fibrosis is

the a cetylated exepolysaccharide composed of B 1,4 linked mannuronic acid and L-guluronic acid. This polymer is responsible for the mucoid nature of Ps. aeruginosa strains isolated from chronically colonized patients with cystic fibrosis A synonymous term is "alginate". Alginate production is the most remarkable virulence trait associated with cystic fibrosis infection caused by Ps. aeruginosa. More than 80% of cystic fibrosis isolates produce alginate, whereas this phenotype is found is no more than 2.5% of other infections (Ohman et al., 1985). Alginate production is an unstable characteristic, even with strains isolated from patients with cystic fibrosis, but the lung appears to select or encourage it. It is likely that alginate production contribute to the pathogenesis of Ps. aeruginosa lung infections in ways similar to those of other polysaccharide capsules (antiphagocytosis, adherence). But more important, the production of this material could contribute to pathogenesis in more novel ways. Alginates in the presence of calcium ferm agel that surrounds the Ps. aeruginosa cells with a matrix that may shelter large numbers (microcolonies) from

host defenses and protect them from some charged antibiotics, although the latter possibility is presently controversial. It has been recently proposed that alginate may induce immune complex like damage in patients with cystic fibrosis (Pier, 1985). Woods and Bryan (1985) using the chronic rat lung model, recently reported that some animals immunized with alginate developed pulmonary immune complex deposits. However, it was not clear whether these complexes contained alginate. These observations, if verified and subsequently applied to Ps. aeruginosa infections in patients with cystic fibrosis add another dimension to the capabillities of Ps. aeruginosa in causing disease. This bacterium would not only be invasive and toxigenic, but pathogenic in a way limited to a few bacterial parasites: induction of immunologic damage.

There are several stages in a protype Ps. aeruginosa infection: adherance, colonization, invasion and dissemination and systemic effects or toxemia. There may also be multiple ramification of each of these stages.

Furthermore, the relative importance of each stage in different types of Ps. aeruginosa infection is variable. For example, invasion is not a feature of Ps. aeruginosa infection in patients with cystic fibrosis. Systemic toxicity is also an uncommon manifestation. Virulence factors are needed to be classified to detect their interaction. For example, what is the effect in vivo of proteolytic enzymes on other proteinaceous virulence factors? Proteases could enhance their activity or could negate their effects (Vasil 1986).

2- Inanimate reservoir:

Most environment is the most important source of inanimate reservoir, contaminated fluid which have caused infections with Ps. aeruginosa include eye drops and ophthalmic solutions (Ayliffe et al., 1966). Also, it was reported in bottle replenished without adequate disinfection, dilute phenolic solution, hand cream steroid cream, incubators, respiratory ventilators, shaving and nail brushes, and clothes. Food has been found to carry Ps. aeruginosa in the kitchens, the same type appearing subsequently in the faeces of patient. (Shooter et al., 1969 and Govan, 1978).

The role of Pseudomonas aeruginosa in epidemiology:

Hospital infection is the result of the transmission of pathogenic organisms to previously uninfected patient from source in the environment of hospital. Infection is almost restricted to patients in hospital infection may be acquired from external sources (Exogenous infection by air - borne route or by contact with human or inanimate vectors or it may be self infection by bacteria present in patient's skin, respiratory tract or intestinal tract (Clarke and Richmond, 1975).

Ps. aeruginosa is a normal inhabitant of human intestinal tract where it is present in small percentage (about 11% of the normal population) (Ringen and Droke, 1952). It increase in the intestine when normal coliform flora has been suppressed by antimicrobial drugs (Yow, 1952). It is found sometimes on normal skin (axilla and perineum) (Hojyo- Tomoka and Richard, 1973). It was isolated from swage barnyards (2.5%) soil 3% and poluted water but not natural water supplies (Ringen and Drake,

1952). Jawetz (1952) did not consider Ps. aeruginosa as a pathogen in the usual sense of the ward and called it opportunist.

Ps. aeruginosa is incriminated in:

1- Urinary tract infection where it is introduced by catheterization or other diagnostic or therapeutic instrumentation (Kass 1955 - McLoad 1958 - Murphy and McDonald 1961).

2- Infected wound (Sussman and Stevens 1960).

3- Infected burns (Graber et al., 1961).

4- Osteomyelitis.

as a complication of puncture of wounds of the foot or haematogenous infection of urethra (Clarke & Richmond, 1975).

5- Acute purulent meningitis:

It follows lumber puncture, spinal an aesthesia, intrathecal medication or hand injury (Botterell and Magner 1945 - Harris et al., 1946).

6- Chronic otitis media (Funk 1901).

Otitis externa (Hall et al., 1968).

7- Respiratory tract infection

Pneumonia (Finland 1960).

Chronic purulant bronchial infections (Pines, 1967 - Darrell and Water Worth 1967).

8- Gastro intestinal ulceration and localised peritonitis (Hubbard et al., 1957).

Ps. aeruginosa may be responsible for typhoid like infections and liver abscess (Pons, 1927). Severe cases of infantile diarrhoea associated with the organism were also recorded (Hunter and Ensign 1947).

9- Septicaemia: It occurs as a complication of malignancy also occur in new born infant (Margartten et al., 1961). Clarke and Richmond (1975) reported that bacterial virulence was enhanced by the possesion of a mucoid envelop which protects the organism against phagocytes and antibiotics.

TYPING OF PSEUDOMONAS AERUGINOSA

Ps. aeruginosa is an important cause of infection in hospital patients (Kominos et al., 1972) and is difficult to control owing to its resistance to many antibiotics (Jacoby 1974 & Eykyn and Phillips 1975). Various methods of typing had been used in epidemiological investigation:

1- Serological typing:

Based on Osomatic antigen was easy to perform and the results were generally reliable (Parker, 1972). Antisera were available commercially (Al-Dujailia and Harris 1974) and were convenient to use but there was no information on their potancy. A disadvantage was that certain serological types predominate.

2- Bacteriophage typing:

Bacteriophage typing requires facilities that might not be available in routine diagnostic laboratories,

although a variety of typing pattern occur, reproducibility was less satisfactory than in staphylococcus phage typing. The combined use of phage typing and serological typing may be of value in subdividing the common serotypes (Parker, 1972).

PYOCINS

Pyocins are the bacteriocins of Ps. aeruginosa i,e antibiotic substances produced by strains of the species which have the characteristic property of being lethal only for other strains of Ps. aeruginosa or closely related species (Govan 1978). Pyocinogeny means the ability to produce pyocin. It was firstly described by Jacop (1954) and is stable characteristic which is normally repressed in most bacterial cells. Govan (1978) indicated that when synthesis is limited, it could be increased by induction with ultraviolet light or Mitomycin C.

Bacteriocins differ from most other antibiotics in that the producer strain is immune to the action of its own bacteriocin which is directed only to bacteria which are closely related to the strain which produce them. Bacteriocin production was found to be regulated by plasmid or chromosomal elements where bacteriocin activity was initiated by adsorption of bacteriocin to specific outer membrane receptors on susceptible cells (Govan,1986).

Pyocins may be classified in two main groups on the basis of their molecular weight. The high molecular weight groups (mol wt 10^6 - 10^7) is composed of two types of particle, the contractile and filamentous both visible in the electron microscope and apparently similar to certain defective bacteriophage. The structure and activity of these pyocins have been described in details by several workers (Kageyama 1964; Higerd et al., 1967; Takeya et al., 1969, Govan 1974a). The other major group of pyocins consist of substance of lower moleculas weight ($C.10^5$), which are not sedimentable by ultracentrifugation and have not been resolved in the electron microscepe, but are apparently simple proteins resembling some colicines (Ito, et al., 1970; Ohkawa et al., 1973). Strains of Ps. aeruginosa are immune to their own pyocin but sensitive cells are killed following attachment of pyocin to specific receptors on the cell surface. These pyocins are known as S type or small. R type of pyocins resemble the tail of contractile phage (Higerd et al., 1967; Govan, 1968, 1974 a) whilst morphologically distinct rod shaped flexuous or F type pyocins resemble the tail of non

contractile Pseudomonas phage (Takeya et al., 1969; Govan, 1974). R type pyocins may show immunological cross reactivity with contractile phages (Ito and Kageyama 1970) and synthesis of pyocin R is directed from a chromosomal locus (Kageyama 1970a). In case of R type pyocin the receptors are lipopolysaccharides (Govan 1978), contraction occurs following to the cell surface and is also observed following attachment of the pyocins to purified lipopolysaccharide from sensitive cells (Govan, 1974a). Kaziro and Tanaka (1965) have shown that in case of pyocin. R cells are most sensitive in the iogarethmic growth phase, synthesis of RNA, DNA and protein is interrupted at the ribosomal level without direct contact with ribosemes.

Five R type pyocins have been reported which are almost identical with one another in their morphology and subunit composition, though distinct in receptor binding specificity Kumazaki and Ishii (1982) isolated fibers from pyocin R_2 - R_3 - R_4 by essentially the same procedure as used in isolation of pyocin R_1 fiber with

unimpaired receptor binding ability. All the pyocins isolated including R_1 fiber were indistinguishable from one another (Kumazaki and Ishii 1982). After adsorption of pyocin R_1 to the outer membrane of a sensitive cell, a pyocin R_1 particle undergoes sheath contraction and core penetration through the outer membrane and causes an increase in permeability of the membrane to hydrophobic solutes (Uratani and Kageyama 1977 - Uratani, 1982). Inhibition of active transport and macromolecular synthesis occurs and finally the cell dies (Kaziro and Tanaka, 1965). On the other hand the membrane electron transfer system is not affected by pyocin R_1 treatment although oxygen uptake for certain substrate is interfered with as a result of inhibition of active transport (Kaziro and Tanaka 1965). In addition, it has been found that a number of pyocin particles added to culture decrease the turbidity of the cell suspension because of cell lysis but the presence of a high concenteration of magnesium represses the pyocin R_1 induced cell lysis (Kaziro and Tanaka 1965 & Uratani and Kageyama 1977). The inhibitory

action of pyocin R_1 on active transport has not been investigated in detail under combined condition in which respiration continues and cell lysis is repressed. Pyocin R_1 caused depolarization of the cytoplasmic membrane in addition to inhibition of active transport. S type pyocins also of low molecular weight (approximately 10^5 Daltons) had been isolated by Ito et al.,(1970).

Pyocins of Ps. aeruginosa:

Bacteriocin production and susceptibility are more commonly observed in strains of Ps. aeruginosa than in most other bacterial species. In addition the classes of bacteriocin like agents produced represent the combined range found in other species. Phage tail like R and F pyocins (Govan 1974 - Kuroda and Kageyama 1983 & Shinomiya 1984) are produced by more than 90 per cent of clinical isolates and colicin like S pyocins are produced by over 70 per cent of strains (Fyfe et al., 1984), susceptibility to these bacteriocins is found in almost 10 per cent of strains. Some strains of Ps. aeruginosa (Govan 1978) also produce a class of low molecular weight,

trypsin resistant bacteriocin resembling the microcins of enterobacteria (Asensio et al., 1976 - Baquero & Mareno, 1984). Ohkawa et al., (1981) reported that the effeciency of killing by pyocin S_2 was very low when the pyocin S_2 susceptible strain Ps. aeruginosa PML 1550 was grown in an iron defecient condition (1 μ M or less), which was necessary to produce a fully sensitive cell population and was accompanied by the appearance of an outer membrane protein which appeared to act as the receptors for pyocin S_2. Competition experiments between pyocin S_2 and culture supernatant containing the yellow green Pseudomonas siderophere, pyoverdin suggest that the pyocin S_2 receptor was not associated with pyoverdin mediated iron assimilation. Studies have expanded these observation on the pyocin susceptibility to other untreated pyocin - Pyocin S a under iron defecient conditions. In addition in competitive growth experiments involving the pyocin Sa producer strain 1003 and 1516, the surival of 1516 decreases in a dose dependant manners as the Fe^{+++} content of the culture medium was reduced. Further studies suggested

that the phenomenon was common in S pyocins but that low iron concenteration did not enhance the killing ability of other classes of pyocins including R and F pyocins and those resembling the microcins. The ubquitous nature of Ps. aeruginosa would allow studies to determine the relative frequency of the different classes of pyocin produced by isolates from various environment and in vitro location. In ecological terms, it is interesting to note that bacteriocinogeny in strains of Ps. aeruginosa appears more frequently in clinical than environmental isolates. Since pyocin production appears to be unaffected by iron concenteration at least in vitro. It is possible that carefully designed in vivo studies might show that susceptibility to pyocins might play a significant role in the establishment of pyocin, producing strains particularly in these infected tissues where the organism was known to grow under iron limitation such as chronically colonized lungs of patients with cystic fibrosis (Brown et al.,1984).

Methods of pyocin typing of Ps. aeruginosa:

Pyocin typing might be accomplished in two ways namely "active" and "passive". Active pyocin typing depends on the pattern of growth inhibition produced by the pyocins of the strain being typed on a set of indicator strains of Ps. aeruginosa. Passive pyocin typing depends on the susceptibility of the strains being typed to the pyocins produced by a set of Ps. aeruginosa strains chosen for this purpose. The most widely used active method is that of Gillies and Govan (1966) and Govan and Gillies (1969). This method has been used extensively in epidemiological studies (Tinne et al., 1967 & Henderson et al., 1969).

Pyocin typing has been the most popular method for typing Ps. aeruginosa in hospital laboratory. Most worker used across streaking method. Indicator strains are streaked across an area of medium that has supported the growth of the test strain to see if it has produced bacteriocin and comparing the pattern of inhibition of growth of indicator strains with standard table of type patterns (Pitt, 1980).

Pyocin typing can be performed with liquid pyocins produced in cultures induced by mitomycin C (Rampling et al., 1975).

An investigation was carried out to determine the value of active and passive pyocin typing in the studing of Ps. aeruginosa infections acquired in hospitals. Active typing was a more reliable and reproducible method than passive typing. Both methods were used in studies of nine outbreaks of infection. In six of these episodes there was good agreement between the two methods. Less clear - cut results were achieved in the remaining three episode. In one of these, active typing gave more valuable information. However, both methods are easy, convenient and of value in epidemiological studies (Falkiner and Kean, 1977).

Hollowey (1960) reported that pyocinogeny was common in strains of Ps. aeruginosa and suggested that pyocin production might prove a useful epidemiological tool of this species. Darrell and Wahba (1964) described across

streaking technique based on the earlier colicin typing method for Escherichia Coli. In 1966 Gillies and Govan described across streaking technique, which had been developed over several years.

Streaking method: (Gillies and Govan, 1966)

The strain to be typed is streaked diametrically across the surface of a nutrient agar plate to give an inoculum width of approximately 1cm. After incubation for 14 - 18 hours at 32°C the growth was removed with microscope slide which has been dipped in chloroform and the remaining viable growth was killed by pouring 3 ml of chloroform into the lid of the petri dish and replacing the medium containing portion for 15 min. The plate was then opened and the traces of chloroform were eliminated by exposing the medium to the air for a few minutes. Cultures of the indicator strain 1 - 8 grown in nutrient broth for 4 hours at 37°C were streaked on to the medium by means of a multiple loop inoculator at right angles to the line of the original inoculum. The plates were then incubated for 18 hours at 37°C.

Any pyocins produced by the original inoculum diffuse into the medium during the first period of incubation and then exert their inhibitory action on the indicator during the subsequent incubation. The pyocin types of the strains under examination were determined from the patterns of inhibition which they produce on eight indicator strains of Govan (1978).

Revised pyocin typing method for Ps. aeruginosa (Fyfe et al., 1984):

This method enables us to significantly improve the speed and application of pyocin typing. In this method, after incubation of the tested strains for 6 hours and exposure to chloroform, the indicator strains were applied in agar overlays without prior removal of the test strain growth, the inhibition pattern of the indicator strains was recorded and pyocin type was determined according to Govan (1978).

Relationship of bacterial enzyme production and pyocin typing:

Strains of Ps. aeruginosa were studied from 100 patients with bacteraemia. In-vitro quantitation of extracellular enzymes (lecithinase, protease and elastase) and pyocin typing of these isolates were performed. No significant difference was found in the quantity of the enzyme produced or in the pyocin types by isolates obtained from patients dying from bacteraemia or surviving this serious infection. Quantitation of the extracellular enzymes and pyocin types of blood isolates were contrasted with similar data obtained from sputum, urine and skin isolates. One third of all strains produced minimal or no extracellular enzymes regardless of their source. However, the highest enzyme-producing strains were observed in the blood isolates. Although a greater variability of pyocin types were found in blood culture isolates, there was no significant difference between the pyocin types found in blood, urine, sputum or skin isolates. The lack of correlation between the in - vitro quantity

of lecithinase, protease and elastase produced by Ps. aeruginosa strains isolated from bacteraemic patients and the prognosis of these patients supports the possible local rather than systemic significance of these extracellulas enzymes in the pathogenesis of Ps. aeruginosa bacteraemia (Balth et al., 1979).

Pyocin sensitivity of Neisseria gonorrhoea and its feasibility as an epidemiological tool:

Pyocin inhibition of Neisseria gonorrhoea and its feasibility as a gonococcal typing scheme were examined. Mitomycin C. induced pyocin lysates of Pseudomonas aeruginosa were able to selectively inhibit the growth of gonococcal strains. The particles associated with the inhibitory activity were non dialyzable, heat labile, protease sensitive, trypsin resistant and of large molecular weight by membrane and gel filtration techniques. The inhibitory activity was shown to be specific by absorption with sensitive and insensitive strains of N. gonorrhoea and Ps. aeruginosa. Partial purification of

pyocin lysates by ammonium sulfate precipitation followed by ultracentrifugation revealed phage like particles consistent with high molecular weight R type pyocins. The particles were associated with increased inhibitory activity and could be seen associated with the gonococcal cell surface. One hundred and six gonococcal strains could be differentiated on the basis of their sensitivity of 23 pyocin extracts. Thirty different patterns of pyocin inhibition were seen. Isolates from different body sites from the same patient could generally be identified as being similar strains, in general agreement between pyocin typing and available epidemiological information (Sidberry and Sadoff, 1977).

ANTIBIOTIC RESISTANCE OF PS. AERUGINOSA

Mechanism of aminoglycoside resistance of Ps. aeruginosa involve either resistance R- plasmid - mediated enzymes that inactivate aminoglycoside or mutation of chromosomal genes affecting the energy requirement for transport of aminoglycosides into the cell (Bryan, 1979). The resistance of Ps. aeruginosa to the majority of antibiotics due to impermeability of the bacterial cell wall and enzymatic inactivation of the antibiotic (Joklick et al., 1980).

The usage of antibiotic is an important factor in the spread of antibiotic resistance by providing selective pressure for the resistance by gene products (Daschner et al., 1978). In hospitalized patients gentamicin resistance in Ps. aeruginosa has been associated with increased exposure to aminoglycosides and other antibiotics. (Ruben, 1978).

The following antibiotics are commonly used for the treatment of Ps. aeruginosa infections:

1- Gentamicin:

It is produced from Micromonospora purpura and is active in vitro against Ps. aeruginosa (Barder and Waterworth, 1966). It's mode of action probably involves the control of protein synthesis on the ribosomal surface (Sonne and Jawetz, 1969). Gentamicin contains several components of which designated G_1, G_1 A and G_2 but isolation, identification and manipulation of further components A, B, B_1 and X have been reported. Gentamicin A has one quarter to one eight the activity of gentamicin C complex against Staphylococcus aureus and Escherichia coli but has no antipseudomonal activity. Gentamicin B. B_1 are more active but their toxicity is similar to gentamicin X. Gentamicin G is a commercial preparation and supplied as sulphate (Garrod et al., 1981). The actual MIC of gentamicin varies from worker to another and that was explained by Garrod and Waterworth (1969) who found that the results depend on the magnesium content of the medium,

magnesium ions are essential for the growth of Ps. aeruginosa and enhance pigment production, also high concenteration of the magnesium ions reduce the susceptibility of these organisms to the antibiotics. Gentamicin is used in the treatment of urinary tract infections caused by Ps. aeruginosa. It is used in the treatment of Ps. aeruginosa pneumonia successfully. It is also used in the treatment of Gram negative septicaemia, in severe sepsis and used for severe abdominal sepsis after surgical operation (Smith 1985).

Toxicity:

It is ototoxic and nephrotoxic but this occurs when high concenteration of gentamicin accumlated in the kidney and in patients with impaired renal function. Nephrotoxicity also occurs when combination of gentamicin and cephalosporins as the two agents act on the same target organ and this is an example of synergic-toxicity. (Garrod et al., 1981).

The recent resistance of gentamicin is due to R-

factors which confers on the previously sensitive strains the ability to synthesize enzymes that destroys aminoglycosides. It is found that the most resistant strains to gentamicin are frequently isolated from urine and from patients who have received the drug (Bryan 1979).

2- **Amikacin:**

It is a modified kanamycin A. It is more active against Pseudomonas than kanamycin but less active and less toxic than gentamicin or tobramycin. Amikacin is little or not affected by enzymes that degrade aminoglycoside, thus it is active against strains that owe their resistance due to enzymatic mechanism. (Price *et al.*, 1974). 85% of *Ps. aeruginosa* strains resistant to gentamicin were susceptible to amikacin (Draser *et al.*, 1976).

3- **Tobramycin:**

It is active against *Ps. aeruginosa* (Klastersky *et al.*, 1974 - Price *et al.*, 1974). It is more active than

kanamycin against the majority of susceptible bacteria. It is more active against gentamicin resistant strains (Garrod et al., 1981). Cross resistance is due to reduction of the aminoglycoside transport into the cell but independent resistance to gentamicin depends on resistance of tobramycin to gentamicin degrading enzymes elaborated by the resistant strains (Garrod et al., 1981).

Toxicity:

It is toxic against eighth nerve and the kidney. The renal toxicity is enhanced by concurrent adminstration of cephalothin (Klastersky et al., 1974). Tobramycin is superior in activity atainst Pseudomonas than gentamicin.

4- Colistin methane sulphonate (colimycin):

It is isolated from culture filterates of B. colistinus, a microorganism obtained from soil by Mc Millan et al., (1962). It is active against Gram negative organisms including Ps. aeruginosa. (Yow 1952).

Toxicity:

Severe toxic effect have not been occured with therapeutic dosage or in animal experiments (Mc Millan et al., 1962).

5- Carbenicillin:

It is a semi-synthetic penicillin with a wide range of activity than ampicillin and cephaloridene against Gram negative bacteria particularly Ps. aeruginosa and most proteus species, Ps. aeruginosa is sensitive but to a relatively high concenteration (Pericival and Leigh 1967). Bodey et al., (1971) found that carbenicillin disodium was effective in the treatment of 91% of the infections of Ps. aeruginosa however 7% of the infections responded partially. The probably action of carbenicillin by inhibition of cell wall synthesis (Sonne and Jawetz 1969).

Toxicity:

Toxic effects are minimal consists mainly of transient elevation in serum glutamic oxalacetic trans-

aminase and serum glutamic pyruvic transaminase level (Bodey et al., 1971).

6- Polymyxin B:

There are peptide antibiotics produced by B. polymyxa (Stanly et al., 1947). It increase the permeability of cell envelope (consisting of cell wall and cytoplasmic membrane), presumbly by combination with the phosphatide of the cell wall (Newton 1956).

Toxicity:

Severe toxic symptoms and signs as nephrotoxicity irritation at the site of injection and some neurotoxic symptoms have been reported on parenteral adminstration of polymyxin B. (Jawetz 1952).

MATERIALS AND METHODS

MATERIALS AND METHODS

Materials:

I- Case material:

This work was done on 235 patients:

100 patients from Zifta and 135 patients from Benha hospital suffering from various septic lesions such as:

1- Septic burns especially grade III burns.

2- Septic wounds especially post operative wounds.

3- Urinary tract infections.

All specimens were collected during October, November and December, 1987.

II- Media:

Nutrient agar, nutrient broth (oxoid) 0.03% cetrimide agar and nutrient gelatin (Cruickshank, et al., 1975).

III- Chemicals:

Chloroform (El-Nasr Company).

IV- Antibiotic sensitivity discs (Biomerieux).

Gentamicin	10 µg/disc.
Tobramycin	10 µg/disc.
Polymyxin B	300 µg/disc.
Amikacin	30 µg/disc.
Cefatoxime	30 µg/disc.
Colimycin	10 µg/disc.
Carbenicillin	100 µg/disc.

V- Indicator strains of Govan for pyocin typing:

The indicator strains of Govan (1978 (G1 - 8) were kindly provided by Dr. Wael El Naggar lecturer of Pharmaceutical Microbiology, Mansoura University, Mansoura, Egypt.

Methods:

1- Collection of specimens:

Swabs were taken from burns and septic wounds while urine samples were taken from cases of urinary tract infection.

II - Isolation and identification of Ps. aeruginosa:

Samples were collected in sterile containers or swabs according to the type of material, then they were cultured as soon as possible on nutrient agar and cetrimide agar and incubated at 37°C for 24 - 48 hours in case of nutrient agar. In case of cetrimide agar 24 - 72 hours. Suspected colonies of Ps. aeruginosa were subcultured on nutrient agar slopes for further identification.

Identification of Ps. aeruginosa strains was carried out according to Bergey's Manual of systematic Bacteriology (1984). In this respect, the isolated strains were tested for their negative gram stain, diffusible pigments, gelatin liquifaction, ready growth at 42°C on ordinary media and oxidase test.

III - Antibiotic sensitivity test:

The method described by Anderson (1970) was adopted, it included:

1- Plate preparation:

Twenty ml of melted cooled nutrient agar were added to each 100 m m plate. The depth of agar will be approximately 6mm. then leave plates to dry before inoculation.

2- Preparation of inoculum:

Five colonies were picked with sterile loop inoculated in 4 ml of nutrient broth in a test tube, then incubated at 37°C for 24 hours. The turbidity of the overnight culture was adjusted by sterile nutrient broth until cloudiness equal to barium sulphate standard. Prepared by adding 0. 5 ml of 0.048 molar barium chloride (11.7 gm of barium chloride (Hitre) to 99.5 ml of 1% sulforic acid.

3- Inoculation:

The plate was flooded with the previous broth culture culture left to dry for 30 minutes in incubator.

IV - Application of antibiotic discs:

The tested antibiotic discs were then applied at

adequate spacing using a forceps formerly dipped in alcohol and flammed. The plates were incubated at 37°C for 18 - 24 hours, then examined for the presence of inhibition zone around discs. The strain was considered as sensitive or resistant according to antibiotic charts.

PYOCIN TYPING OF PS. AERUGINOSA

The method of Gillies and Govan (1966) as modified by Govan (1978) was adopted.

The strain to be typed is streaked diametrically across the surface of a nutrient agar plate to give an inoculum width of approximately 1Cm. After overnight incubation for 14 - 18 hours at 32°C the growth was removed with a microscope slide which has been dipped in chloroform and the remaining viable cells were killed by pouring 3 ml of chloroform into the lid of Petri dish and replacing the medium containing portion for 20 minutes. The plate was then opened and the traces of chloroform were eleminated by exposing the medium to the air for a few minutes. Cultures of the indicator strains 1 - 8 grown in nutrient broth for 4 hours at 37°C were streaked on to the medium by means of a sterile loop at right angles to the line of the original inoculum. The plates were then incubated for 18 hours at 37°C.

Any pyocins produced by the original inoculum diffuse into the medium during the first period of incubation

and then exert their inhibitory action on the indicator strains during subsequent incubation. The pyocin types of the strains under examination were determined from the patterns of inhibition which they produce on the eight indicator strains of Gillies and Govan (1966) (Table 2).

Table (2) Inhibition patterns of 105 pyocin types (pyotypes) of Govan and Gillies method (active pyocin typing method).

Pyocin type	Inhibition pattern							
	1	2	3	4	5	6	7	8
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	–
3	+	+	+	+	+	+	–	+
4	+	+	+	+	+	+	–	–
5	+	+	+	+	+	–	+	+
6	+	+	+	+	+	–	+	–
7	+	+	+	+	+	–	–	+
8	+	+	+	+	+	–	–	–
9	+	+	+	+	–	+	+	+
10	+	+	+	+	–	+	+	–
11	+	+	+	+	–	+	–	+
12	+	+	+	+	–	+	–	–
13	+	+	+	+	–	–	+	+
14	+	+	+	+	–	–	+	–
15	+	+	+	+	–	–	–	+
16	+	+	+	+	–	–	–	–
17	+	+	+	–	+	+	+	+
18	+	+	+	–	+	+	+	–
19	+	+	+	–	+	+	–	+
20	+	+	+	–	+	+	–	–
21	+	+	+	–	+	–	+	+
22	+	+	+	–	+	–	+	–
23	+	+	+	–	+	–	–	+
24	+	+	+	–	+	–	–	–
25	+	+	+	–	–	+	+	+
26	+	+	+	–	–	+	+	–
27	+	+	+	–	–	+	–	+
28	+	+	+	–	–	+	–	–
29	+	+	+	–	–	–	+	+
30	+	+	+	–	–	–	+	–
31	+	+	+	–	–	–	–	+
32	+	+	+	–	–	–	–	–
33	+	+	–	+	+	+	+	+
34	+	+	–	+	+	+	+	–
35	+	+	–	+	+	+	–	+
36	+	+	–	+	+	+	–	–
37	+	+	–	+	+	–	+	+
38	+	+	–	+	+	–	+	–
39	+	+	–	+	+	–	–	+
40	+	+	–	+	+	–	–	–
41	+	+	–	+	–	+	+	+
42	+	+	–	+	–	+	+	–
43	+	+	–	+	–	+	–	+
44	+	+	–	+	–	+	–	–
45	+	+	–	+	–	–	+	+
46	+	+	–	+	–	–	+	–
47	+	+	–	+	–	–	–	+
48	+	+	–	+	–	–	–	–
49	+	+	–	–	+	+	+	+
50	+	+	–	–	+	+	+	–
51	+	+	–	–	+	+	–	+
52	+	+	–	–	+	+	–	–
53	+	+	–	–	+	–	+	+
54	+	+	–	–	+	–	+	–
55	+	+	–	–	+	–	–	+
56	+	+	–	–	+	–	–	–
57	+	+	–	–	–	+	+	+
58	+	+	–	–	–	+	+	–
59	+	+	–	–	–	+	–	+
60	+	+	–	–	–	+	–	–
61	+	+	–	–	–	–	+	+
62	+	+	–	–	–	–	+	–
63	+	+	–	–	–	–	–	+
64	+	+	–	–	–	–	–	–
65	+	–	+	+	+	+	+	+
66	+	–	+	+	+	+	+	–
67	+	–	+	+	+	+	–	+
68	+	–	+	+	+	+	–	–
69	+	–	+	+	+	–	+	+
70	+	–	+	+	+	–	+	–
71	+	–	+	+	+	–	–	+
72	+	–	+	+	+	–	–	–
73	+	–	+	+	–	+	+	+
74	+	–	+	+	–	+	+	–
75	+	–	+	+	–	+	–	+
76	+	–	+	+	–	+	–	–
77	+	–	+	+	–	–	+	+
78	+	–	+	+	–	–	+	–
79	+	–	+	+	–	–	–	+
80	+	–	+	+	–	–	–	–
81	+	–	+	–	+	+	+	+
82	+	–	+	–	+	+	+	–
83	+	–	+	–	+	+	–	+
84	+	–	+	–	+	+	–	–
85	+	–	+	–	+	–	+	+
86	+	–	+	–	+	–	+	–
87	+	–	+	–	+	–	–	+
88	+	–	+	–	+	–	–	–
89	+	–	+	–	–	+	+	+
90	+	–	+	–	–	+	+	–
91	+	–	+	–	–	+	–	+
92	+	–	+	–	–	+	–	–
93	+	–	+	–	–	–	+	+
94	+	–	+	–	–	–	+	–
95	+	–	+	–	–	–	–	+
96	+	–	+	–	–	–	–	–
97	+	–	–	+	+	+	+	+
98	+	–	–	+	+	+	+	–
99	+	–	–	+	+	+	–	+
100	+	–	–	+	+	+	–	–
101	+	–	–	+	+	–	+	+
102	+	–	–	+	+	–	+	–
103	+	–	–	+	+	–	–	+
104	+	–	–	+	+	–	–	–
105	+	–	–	+	–	+	+	+

+ = inhibition – = no inhibition

Selection of the new pyocin typing set:

Of the 50 isolated strains of Ps. aeruginosa, 8 strains were selected on the basis of antibiotic resistance for study to develop a set of indicator strains for pyocin typing. These indicators set were used to type randomly selected 20 strains six times at time interval of one week. Comparison was done simultaneously between the proposed set of pyocin typing and Govan's one taking in consideration, reproducibility, discriminatory ability and percent typability. The last two items were determined when all the tested 50 isolated strain were subjected to typing by both sets of pyocin typing.

Reproducibility value:

Reproducibility value for an individual strain was obtained by dividing the number of cultures in which repeated tests yielding exactly the same results by the total number of cultures tested against that indicator strain multiplied by 100 to obtain percentage (Schable et al., 1986).

i.e.,

Reproducibility value:

$$= \frac{\text{number of cultures in which repeated tests yielding exactly the same number}}{\text{The total number of cultures tested against that indicator strain.}} \times 100$$

Discriminatory ability: It was defined as the number of pyocin types that represented 50% of the isolates tested (Schable et al., 1986).