PYOCINE TYPING OF PSEUDOMONAS AERUGINOSA STRAINS OF AIN SHAMS UNIVERSITY HOSPITALS

THESIS

Submitted in Partial Fulfilment For the
Master Degree in
Clinical Pathology

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1985

بسم الله الرحمن الرحيم

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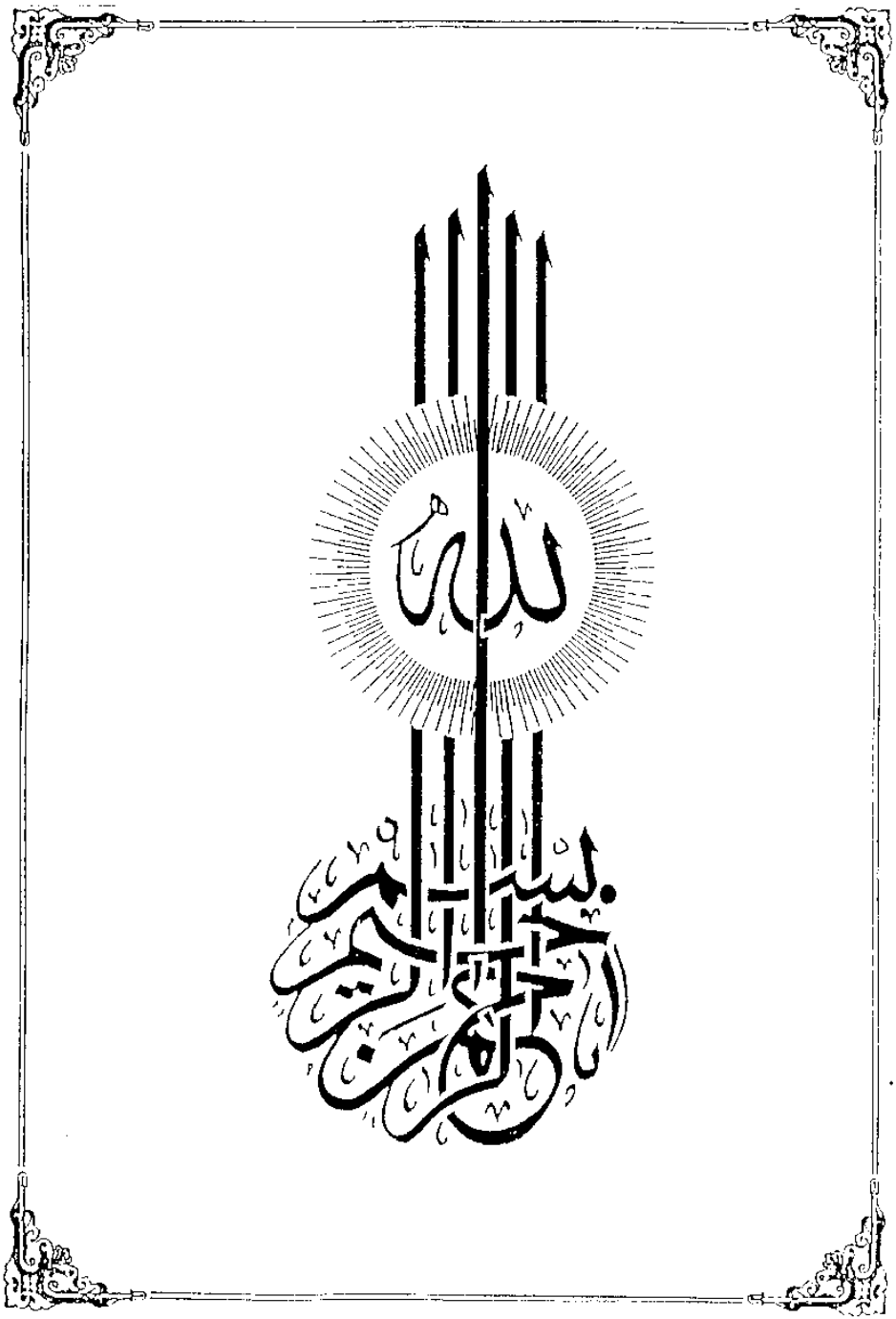
سورة الإسراء (آية ٨٥)



ACKNOWLEDGEMENT

It is a great pleasure to express my sincere thanks and gratitude to professor Dr. Iglal Hassan El-Falaky, professor of clinical pathology, Ain Shams University. She was ever by her enthusiastic guedance, innovation, constructive criticism and wise advice. Not a word escaped her scrutiny. With everlasting patience she revised the initial manuscripts and contributed to its final form. Her continuous encouragement made this work possible.

I wish to acknowledge with profound gratitude the cooperative collaboration and purposeful ahead of Dr. Ibrahim Khalil Aly, Lecturer of clinical pathology, Ain Shams University.

I am indebted to all member of Clinical Pathology Department, Ain Shams University for their kindness and contribution in this study.

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INTRODUCTION

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Pseudomonas aeruginosa has assumed an important role as a causative organism in hospital epidemics and an increasingly frequent cause of death from bacterial sepsis (Alder and Finland, 1971). Protracted hospital outbreaks of urinary tract infection and bacteremia due to multiply resistant gram negative bacilli have become an increasingly common and serious problem (Stamm et al., 1980).

The incidence of nosocomial infections and antimicrobial resistance rates of nosocomial pathogens vary considerably among Countries and even among intensive care units within one hospital. Such differences might be due to antibiotics. Since intensive care patients are given more antimicrobials than any other group of patients (Daschner et al., 1980).

Pseudomonas aeruginosa is a frequent contaminant of respiratory apparatus, humidifier, drainage bottle, antiseptic solutions, hand creams, anaesthesiology and inhalation equipment (Cain et al., 1980). Patients with uncontrolled diabetes, renal failure, malignancy and those receiving high doses of corticosteroids, cytotoxic drugs and immunosuppressives are more liable to P. aeruginosa infection (Heckman et al., 1972).

With the rising frequency of P. aeruginosa infection and the increasing numbers of patients with low resistance, There is a need for reliable typing procedure for P. aeruginosa (Heckman et al., 1972). Various methods had been done for typing isolates and finding out the source of infection as serotyping by (Al Dujaili and Harris, 1974). Phage typing by (Postic and Finland, 1961) and pyocine typing by (Darrell and Wahba, 1964). Serotyping which depends on somatic antigen is easy to perform and the results are generally reliable (Parker, 1972). Phage typing requires facilities that may not be available in all routine diagnostic laboratories and its result is less satisfactory than in staphylococcus phage typing (Falkiner and Keane, 1977). Phage typing in P. aeruginosa should always be combined with serological typing (Bergan and Kozaczec, 1979). Pyocine typing is a simple reliable and reproducible method of typing P. aeruginosa for epidemiological purposes (Jain et al., 1981). It may be accomplished in two ways, active and passive methods (Falkiner and Keane, 1977). Active pyocine typing depends on the pattern of growth inhibition produced by the pyocines of the strains being typed on a set of indicator strains of P. aeruginosa (Gillies and Govan, 1966). Passive typing depends on the susceptibility of the strains being typed to the pyocines produced by a set of P.aeruginosa strains chosen for this purpose (Bergan, 1979).

Discrimination of P.aeruginosa strains had been improved by the use of combined typing methods such as serological and pyocine typing (Wahba, 1965), serological and phage typing (Shooter et al., 1969), Lastly pyocine production and pyocine sensitivity (Farmer and Herman, 1969 ; Tagg and Mushin, 1973)

Aim of the work :

Pyocine typing is done to find out the prevalence of different pyocine type strains causing infection in Ain Shams University Hospitals.

REVIEW OF LITERATURE

PSEUDOMONAS AERUGINOSA

The genus Pseudomonas comprises more than 140 species and these species are widely distributed in nature as saprophytes and plant pathogens. Only few species among which P. aeruginosa can cause disease in man (Wahba and Darrell, 1968).

Pseudomonas aeruginosa was first isolated from blue pus by Gessard, (1882) who named it bacillus pyocyaneus.

Morphological characteristics :

Pseudomonas aeruginosa are gram-negative, non-sporing rod, motile with one polar flagellum, some strains may have fimbriae and some are capable of slime production (Liu et al., 1967). Ultrastructural features show that the lipopolysaccharide present in the cell wall determines serologic specificity and susceptibility to bacteriocine (Pyocine) as the pyocine activity is associated with protein component of lipopolysaccharide-protein complex of the cell wall. The somatic or O-antigenicity of P.aeruginosa had been associated with this complex (Bergan, 1973).

Cultural characteristics :

Most strains of P.aeruginosa are aerobes but can grow slowly in an anaerobic environment if nitrate is present as a

hydrogen acceptor. Optimal temperature for growth is 37°C but P.aeruginosa can survive and multiply over a wide temperature range (20 - 42°C). Growth at 42°C had been considered to be one of the most reliable features distinguishing P.aeruginosa from other Pseudomonas species (Wahba and Darrell, 1965; Heckman et al., 1972). It grows readily on a wide variety of culture media. On nutrient agar most strains produce diffusible greenish, yellowish or brown pigment which colours the colonies slightly and the surrounding medium intensely (Heckman et al., 1972). On blood agar the organism produces complete haemolysis and the pigment production is usually not obvious (Wahba and Darrell, 1965). It can grow on MacConkey agar medium producing pale yellow colonies being non-lactose fermenter. In broth it gives rise uniform turbidity and surface pellicle.

The organism grows well on ordinary media and 24 - 48 hours colony is variable in character. Six colonial types of P.aeruginosa were observed, S, SF, F, mucoid, gelatinous and dwarf type, the latter needs 48 hours incubation (Wahba and Darrell, 1965). There was considerable instability in colonial form on subculture. This was particularly with the gelatinous type which tends to lose many characteristics but retains its firm adherence to the medium (Schultz, 1947).

Cetrimide agar was considered a useful selective medium for the isolation of P.aeruginosa from pus and sputum (Lowbury and Collins, 1955). On improved cetrimide agar (Brown and Lowbury, 1955) colonies of P.aeruginosa show brilliant yell · fluorescence.

Pigment production by Pseudomonas aeruginosa :

Pseudomonas aeruginosa produces variable number of exopigments including :

1- Pyocyanin (blue pigment) :

Aphenazine pigment forred in peptone media. It is soluble in both chloroform and water (Heckman et al., 1972). Its demonstration requires a balance of magnesium, iron and other ions. Modified sierra media containing magnesium ion were used for its enhancement (Wahba and Darrell, 1965).

2- Fluorescin (yellow pigment) :

Formed only in the presence of phosphate and is soluble in water but not chloroform. It is not unique to P.aeruginosa It fluoresces under ultraviolet light (Wahba and Darrell, 1965). The combination of pyocyanin and fluorescin produces a bright green colour that diffuses throughout the redium.

3-Pyomelanin :

A dark brown pigment formed in some old cultures, possibly a derivative of fluorescin (Young, 1947).

4-Pyorubin :

A red pigment which is formed by some strains. It is water soluble and chloroform insoluble (Meader, 1924).

Various products of Pseudomonas aeruginosa :

Pseudomonas aeruginosa produces group of exotoxins that might determine its pathogenicity. They include : haemolysin, exotoxin A, proteolytic enzymes, enterotoxin, pigments as well as bacterial slime (Liu, 1964). Also it produces lecithinase and lipase (Wretlind et al., 1971). These powerful extracellular toxins can produce leucopenia, acidosis, circulatory collapse, pulmonary oedema and tubular necrosis (Stone, 1966).

1- Haemolysins :

Pseudomonas aeruginosa produces two haemolytic substances one is heat labile and appears to be phospholipase, the other substance is heat stable glycolipid of low toxicity. The production of haemolytic substances requires high carbohydrate and low phosphate content of the environment (Liu, 1964). Haemolysin production in vitro may be a suitable marker of virulence (Al-Dujaili and Harris, 1978).

2- Enterotoxin :

Pseudomonas aeruginosa had been associated with diarrheal conditions described as five days fever or necrotising enteritis in the tropics. These are due to elaboration of an enterotoxin (Liu, 1964).

3- Exotoxin A :

A protein of more than 10,000 daltons. If injected intravenously its LD 50 for mice is approximately 7 ug (Chung and Collier, 1977) while intradermal injection causes oedema and necrosis and in intraperitoneal injection 5-10 LD 50 causes leucopenia and death. Autopsy show necrosis of the liver, oedema, haemorrhage of the lungs and kidney, its action appears to be as diphtheria toxin (Leppla et al., 1978).

4- The slime :

The slime of P.aeruginosa probably functions like the capsule of Pneumococci in preventing phagocytosis by the host and enhances the virulence of the organism. However it differs from that of pneumococci in being loosely bound to the surface of P.aeruginosa (Liu et al., 1961).

Mucoid colonies were usually non-pigmented and resembling colonies of Klebsiella, from which they may be distinguished by the Kovacs oxidase test (Wahba and Darrell, 1965).