بسم الله الرحمن الرحيم
جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم
قسم
نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
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15-25- c and relative humidity 20-40%
شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم
بعض الوثائق الأصلية تالفة
SUSCEPTIBILITY OF MULTIRESISTANT NOSOCOMIAL ISOLATES OF PSEUDOMONAS AERUGINOSA TO TROVAFLOXACIN (A NEW QUINOLONE) AND THEIR TYPING BY SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE).

Thesis
Submitted to the Faculty of Medicine,
University of Alexandria
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The Master Degree in
Medical Microbiology

By

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Dedicated to

My dear mother, my great father and my helpful husband for their love and support. I am grateful to all of them. Their love and encouragement were the best support I ever needed throughout my work.
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<table>
<thead>
<tr>
<th>Term</th>
<th>Abbreviation</th>
<th>Description</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Lethal Dose</td>
<td>LD50</td>
<td>Lipopolysaccharide</td>
<td>LPS</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>CF</td>
<td>Minimum Bactericidal Concentration</td>
<td>MBC</td>
</tr>
<tr>
<td>Deoxyribonucleic acid</td>
<td>DNA</td>
<td>Mega base</td>
<td>Mb</td>
</tr>
<tr>
<td>Elongation factor 2</td>
<td>EF-2</td>
<td>Minimum inhibitory concentration</td>
<td>MIC</td>
</tr>
<tr>
<td>Exotoxin A</td>
<td>ETA</td>
<td>Molecular weight</td>
<td>MW</td>
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<tr>
<td>Food and drug administration</td>
<td>FDA</td>
<td>Nicotinamide adenine dinucleotide</td>
<td>NAD</td>
</tr>
<tr>
<td>Gram negative/positive</td>
<td>G-ve/+ve</td>
<td>Phospholipase C</td>
<td>PLC</td>
</tr>
<tr>
<td>International Antigenic Typing Scheme</td>
<td>IATS</td>
<td>Pseudomonas aeruginosa</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>Kilo base</td>
<td>kb</td>
<td>Pulsed-field gel electrophoresis</td>
<td>PFGE</td>
</tr>
<tr>
<td>Kilo Dalton</td>
<td>kDa</td>
<td>Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis</td>
<td>SDS-PAGE</td>
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</table>

Table 1. List of abbreviations.
INTRODUCTION
PSEUDOMONADS

GENUS DEFINITION

All Pseudomonads are aerobic Gram-negative bacilli, motile with polar flagella, mono- or multitrichous, oxidase positive, and catalase positive. Some species can grow anaerobically using nitrate as an alternative electron acceptor. They are non-sporing and not acid fast. Typically, the breakdown of carbohydrates is oxidative. Oxidation of sugars yields relatively small amounts of acids, and oxidation is best detected in ammonium-salts medium, in which the sugar is the only carbon source. Many species produce characteristic water soluble pigments. Some strains are pathogenic for humans and animals. The G+C content of their DNA is 57-70 mol% and the type species is Pseudomonas aeruginosa (1).

P. aeruginosa is frequently present in small numbers in the normal intestinal flora and on the skin of humans and is the major pathogen of the group. Other Pseudomonads infrequently cause disease. The classification of Pseudomonads is based on rRNA/DNA homology and common culture characteristics (Table 2).

Burkholderia pseudomallei causes melioidosis, an endemic glanders-like disease of animals and humans, primarily in Southeast Asia and northern Australia.

Burkholderia mallei causes glanders, a disease of horses, mules, and donkeys transmissible to humans.

Stenotrophomonas maltophilia is the most widely accepted name for the organism previously called Pseudomonas maltophilia and Xanthomas
*maltophilia. Stenotrophomonas maltophilia* is an increasingly important cause of hospital-acquired infections in patients who are receiving antimicrobial therapy and in immunocompromised patients (2).

<table>
<thead>
<tr>
<th>rRNA HOMOLOGY GROUP AND SUBGROUP</th>
<th>GENUS AND SPECIES</th>
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<tbody>
<tr>
<td>I. Fluorescent group</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas fluorescens</td>
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<tr>
<td></td>
<td>Pseudomonas putida</td>
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<td></td>
<td>Pseudomonas stutzeri</td>
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<td></td>
<td>Pseudomonas mendinocina</td>
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<tr>
<td>Nonfluorescent group</td>
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<tr>
<td>II</td>
<td>Burkholderia pseudomallei</td>
</tr>
<tr>
<td></td>
<td>Burkholderia mallei</td>
</tr>
<tr>
<td></td>
<td>Burkholderia cepacia</td>
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<td></td>
<td>Burkholderia picketi</td>
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<td>III</td>
<td>Comamonas species</td>
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<td></td>
<td>Acidovorax species</td>
</tr>
<tr>
<td>IV</td>
<td>Brevundimonas species</td>
</tr>
<tr>
<td>V</td>
<td>Stenotrophomonas maltophilia</td>
</tr>
</tbody>
</table>

**Table 2.** Classification of some of the medically important *Pseudomonads* (2).
PSEUDOMONAS AERUGINOSA

The organism known today as *Pseudomonas aeruginosa* (Latin: full of copper green rust) was earlier called *Bacillus pyocyaneus* (Greek: blue pus) and later *Pseudomonas pyocyanea* because of the characteristic blue-green coloration formed by cultures.

The organism was first isolated in pure culture by Gessard (3) and its pathogenicity was established independently by Ledderhose and Charrin (cited by Bulloch)(4).

*P. aeruginosa* is the epitome of an opportunistic pathogen of humans. It is pathogenic only when introduced into areas devoid of normal defences e.g. when mucous membranes and skin are disrupted by direct tissue damage or when intravenous or urinary catheters are used. It is the most common Gram-negative bacterium found in nosocomial infections, where it is responsible for 16% of nosocomial pneumonia cases (5), 12% of hospital acquired urinary tract infections, 8% of surgical wound infection (2), and 10% of blood stream infections (6).

Immunocompromised patients, such as neutropenic cancer and bone marrow transplant patients, are particularly susceptible to opportunistic infection. In this group of patients, *P. aeruginosa* is responsible for pneumonia and septicaemia with attributable deaths reaching 30%(7).

*P. aeruginosa* is also one of the most common and lethal pathogens responsible for ventilator-associated pneumonia in intubated patients, with directly attributable death rates reaching 38%(8).
In burn patients, although *P. aeruginosa* septicaemia has declined as a result of better wound treatment, outbreaks in burn units are still associated with high (60%) death rates (9).

In the expanding AIDS population, *P. aeruginosa* bacteraemia is associated with 50% of deaths. Cystic fibrosis (CF) patients are susceptible to *P. aeruginosa*, which is responsible for high rates of illness and death in this population (10).

The combination of weakened host defences, bacterial resistance to antibiotics and the production of extracellular bacterial enzymes and toxins (virulence factors) are major factors which contribute to the pathogenesis.

**HABITAT**

Costerton and Anwar called *P. aeruginosa* the most abundant life on earth. It shows a predilection for growth in moist environments, a reflection for its natural existence in soil and water. The major source of *P. aeruginosa* in surface waters is sewage. Natural carriage of *P. aeruginosa* by humans is infrequent. In healthy subjects in the community, faecal recovery may range from 1-15%. However, faecal isolation frequencies of *P. aeruginosa* increase dramatically in patients in hospitals. Rates up to 60% have been recorded (11).

**MORPHOLOGY AND CELL STRUCTURE**

The cells of *P. aeruginosa* are Gram-negative (Figure 1), non-sporing rods; motile, usually by means of a single polar flagellum (Figure 2). They are arranged singly, in small bundles or short chains. Fimbriae may be present and are usually polar and non-heamagglutinating.