

CASE STUDY

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“Vietnamese time bomb.” Pseudomonas, we didn’t start the fire ,a gram negative Non fermentative bacilli ,works on blue. [Resistance pattern observed in Sree Mookambika Institute of Medical Sciences, Kulasekharam which varies from Hospital to Hospital (24-4-2020 to 7-4-2021)]a study.

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Abstract

1. Proteobacteria groups: Gamma group –Pseudomonas 2021 ICD-10-CM Diagnosis Code B96.5 Pseudomonas (aeruginosa) (mallei) (pseudomallei) as the cause of diseases classified elsewhere .B96.5 is a billable/specific ICD-10-CM code that can be used to indicate a diagnosis for reimbursement purposes.
2. Pseudomonads are aerobic gram negative bacilli belong to class gammaproteobacteria. Ubiquitous in soil ever moist environment, wash cloth, swimming pool ,hot tub and contact lens solutions.
3. Its inherent resistance to a wide range of antimicrobial agents so rarely casuse disease.
4. Has fimbriae and other adhesins and several virulence factors .Toxins and enzymes like lipid A in cellwall triggers fever ,vasodilation ,inflammation shock and other symptoms.
5. A mucoid polysaccharide capsule and its role in cystic fibrosis patients & protects from phagocytosis and in burns victims.
6. The ability of pseudomonas to form biofilm by metabolizing the many drugs and pump them out through antiports.²

Keywords: type III secretion, antibiotic resistance, Pseudomonas, biofilm, pyoverdine, swarming

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1 | INTRODUCTION

The pseudomonads umbrella term¹⁶ for pseudomonas are aerobic rod-shaped, gram-

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negative bacteria, motile by means of one or more polar flagella. They do not form spores. They have an absolute aerobic metabolism and the catalase positive. When aerobic bacteria such as *Pseudomonas aeruginosa* and facultative 'coliforms' grow in oxygen, glucose is completely metabolized by aerobic respiration, using it as the final electron acceptor: $\text{Glucose} + 6\text{O}_2 \rightarrow 6\text{H}_2\text{O} + 6\text{CO}_2 \Delta G_0 = -686 \text{ kcal/mole.}^{15}$

The guanine and cytosine (G+C) content of the DNA ranges from 57 -70 mol%. *B. pseudomallei* (formerly *Pseudomonas pseudomallei*) causes melioidosis (pneumoenteritis) in humans. Latent infections may become active some years later due to immunosuppression. The disease is found mainly in Malaysia, Vietnam, Thailand, Myanmar, Guam, Sri Lanka, northern Australia, and the Philippines, especially in rice-growing areas and bacteria enter open wounds but can also be inhaled. *B. pseudomallei* is a highly infectious pathogen (Hazard Risk Group 3), therefore handle specimens with care. It is a small, motile, Gram negative rod which shows bipolar staining (like safety pins), particularly when stained with methylene blue or Giemsa stain.¹³

2 | PROTEOBACTERIA GROUPS:

Gamma group – *Chromatium*, *Francisella*, *Xanthomonas*, *Coxiella*, *Legionella*, ***Pseudomonas***, *Acinetobacter*, *Moraxella*, *Vibrio*, *Aeromonas*, *Haemophilus*, the Enterobacteriaceae + others¹ However, in spite of the progress, the results of these studies suggested that the species assigned to the genus *Pseudomonas*, a number considerably smaller than years before. The number of *Pseudomonas* species to be included in the new edition of Bergey's

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Manual of systematic bacteriology will be about 65 that the list of species should not go beyond January 2000.⁹ The strains of *Pseudomonas syringae* and *P. fluorescens* are the first genetically engineered microorganisms to be released legally in the USA were from which the ice nucleation gene was deleted to competitively exclude indigenous ice nucleation-active bacteria involved in frost damage¹¹.

2] Chang et al. 2001, high densities of *Pseudomonas aeruginosa* living in the insole allow for inoculation into the weight bearing area of the foot if a nail penetrates the foot through the shoe. Fisher et al. 1985; Niall et al. 1997 studied the most common atypical pathogen in this context, and a paradigm for the interaction between environmental compromise, would be the high incidence of osteochondritis to osteomyelitis, caused by *Pseudomonas aeruginosa* in puncture wounds of the foot associated with the wearing of training shoes (sneakers).⁶ In 1983, Pappas et al. reported an outbreak resulting from contaminated flexible fiberoptic bronchoscopes involving 72 patients with positive cultures. Bronchoscopes had initially been disinfected with glutaraldehyde. Matthews and Fitzsimmons 1964; Shooter et al. 1966 the problem was eventually traced to puncture of the suction channel of the bronchoscopes, resulting in contamination of the bronchoscope interior with *Pseudomonas aeruginosa*.⁸ Together with the genera *Burkholderia* and *Stenotrophomonas*, the species of *Pseudomonas* are the most opportunistic pathogens causing infections in hospitalized patients. Plasmid-determined resistance to mercuric ions (Hg²⁺) is very common in *Pseudomonas* spp found in soil and water (Bale et al. 1987). Kahyaoglu et al. 1995, a more recent potential hazard that has been highlighted is *pseudomonas* infection following birth in water baths & *Burkholderia* (*Pseudomonas*) *cepacia*, and *Burkholderia pickettii*, as hospital opportunists. *B. cepacia* grows in water and in the presence of aqueous chlorhexidine and quaternary ammonium compounds and may cause infections in neonates. Shah et al. 1999, *Pseudomonas aeruginosa* is rarely reported from otogenic abscesses despite its presence in the external ear canal of patients with chronic ear disease, a cause of meningitis and brain abscess in a premature baby, *Pseudomonas aeruginosa* is rarely reported from otogenic abscesses de-

spite its presence in the external ear canal of patients with chronic ear disease, a cause of meningitis and brain abscess in a premature baby. Gellin et al. 1997, the occurrence of *Pseudomonas aeruginosa*, is on the increase both in postoperative cases and in injecting drug users; up to 37 percent of isolates. The increase in infections in injecting drug users is represented in most series (Tunkel and Pradhan 2002, Nussbaum et al. 1992) and may account for up to 40 percent. The disease is often necrotizing and there is diffuse bilateral consolidation on chest radiograph with occasional multiple abscesses.

3 | MULTI DRUG RESISTANCE :

Gentamicin and carbenicillin can be effective treatment combination of acute infections.³ Some *B. cepacia* genomovars worsen prognosis of CF patients, (Jones et al. 2001). It is intrinsically highly resistant to antibiotics, including those effective against *P. aeruginosa* but it is usually sensitive to trimethoprim–sulfamethoxazole and chloramphenicol. Wilkinson and Pitt 1995, *Stenotrophomonas (Xanthomonas) maltophilia* also colonizes with CF patients and can result in disease, it does not adversely affect prognosis.⁵ These resistance characters may be transferred to other *Proteus* strains, -to enterobacteria & to *Pseudomonas aeruginosa*. Unfortunately monotherapy can result in the spread of resistant organisms, e.g. *P. aeruginosa*, and this has been demonstrated following the use of ceftazidime (Pedersen et al. 1986). Chartrand and Marks 1994, Emergence of *P. aeruginosa* resistance is also after monotherapy with imipenem and aztreonam. The use of oral quinolones has more flexible management in exacerbations. Ramsey et al. 1999 the use of aerosolized tobramycin given by nebulizer has been shown to eradicate *P. aeruginosa* and improve pulmonary function tests (Smith et al. 1989), but resistance is a problem.[Giakkoupi et al. 2003] Carbapenem resistance in *Pseudomonas*, and some Enterobacteriaceae is becoming an increasing. Two types of class B metallo b-lactamase are prevalent: Verona imipenemase (VIM) and IMP and a VIM-producing *P. aeruginosa* recently spread among 200 patients in Greece

Resistance pattern observed in Sree Mookambika Institute of Medical Sciences, Kulasekharam

which varies from Hospital to Hospital (24-4-2020 to 7-4-2021)

24-4-2020	Netilmycin sensitive	Ertapenam sensitive					
24-4-2020	Netilmycin sensitive	Ertapenam sensitive	Novobiocin sensitive				
11-5-2020	Netilmycin sensitive	Ertapenam Resistant			ceftriaxone Resistant		
23-5-2020	Netilmycin sensitive	Ertapenam resistant Stopped					
25-6-2020	Netilmycin sensitive	Meropenam-sensitive	Aztreonam sensitive-pus culture				
26-6-2021	Netilmycin sensitive	Meropenam sensitive	Aztreonam sensitive-sputum culture				
26-6-2021				Piperacin tazobactam-resistant-urine culture			
13-6-2021 - 30-6-2020				Pit-sensitive-pus and sputum culture			
30-7-2020 - 24-9-2020			Nitrofurantoin resistant-urine culture		Ceftriaxone resistant	Ofloxacin sensitive Urine culture	Polymyxin b sensitive
25-9-2020						Imipenem sensitive	
1-2-2021- 5-2-2021			nitrofurantoin resistant-urine culture				
13-2-21- 28-2-21				Polymyxicin Sensitive	clotrimazole sensitive	Teicoplanin sensitive then resistance	polymyxin b sensitive
6-3-21			Nitrofurantoin sensitive				
20-3-21 - 24-3-21	Norfloxacin sensitive - urine sample	Ofloxacin sensitive					
8-3-1-2021 - 7-4-2021					Clotrimazole sensitive on	Teicoplanin resistance	

4 | CYSTIC FIBROSIS:

Burkholderia (*Pseudomonas*) cepacia is a lysine positive, that can be distinguished from *Pseudomonas* spp opportunistic pathogen in being of cystic fibrosis patients.³ *Pseudomonads* found in bottled waters at densities ranging from 10³ to 10⁵ organisms per milliliter reported in some drinking-water supplies include *Pseudomonas aeruginosa*, *Burkholderia cepacia* (*Pseudomonas cepacia*), *Pseudomonas fluo-*

rescens, Burkholderia mallei (Pseudomonas mallei), Stenotrophomonas maltophilia (Pseudomonas maltophilia), Pseudomonas putida, and Comamonas testosteroni (Pseudomonas testosteroni), also Pseudomonas stutzeri, Brevundimonas diminuta (Pseudomonas diminuta), and Delftia acidovorans (Pseudomonas acidovorans). Alginate interferes with antibody coating, inhibits phagocytosis of *P. aeruginosa*. Pseudomonas elastase increases the perme-

ability of epithelial cells, and induces shedding of epithelial cell-surface heparan sulfate proteoglycans, encouraging colonization. The pigments pyocyanin and 1-hydroxyphenazine, the hemolysin rhamnolipid interferes with mucociliary transport. In CF patients produce markedly elevated elastase produced by *P. aeruginosa* interferes with their activity, so that they are unable to interact with receptor sites on pulmonary macrophages or neutrophils⁵ encourages persistent colonization.

6] Biofilm:

Biofilms begin with a single bacterium attaching by binary fission and ultimately a glycocalyx for environmental protection. **Quorum Sensing** is bacteria within a biofilm produce small molecules, such as homoserine lactones, which are taken up by adjacent bacteria and functionally serve as a colony, informing individual bacteria to turn on certain genes at a particular time and these signals are known as quorum sensors.¹⁷ The genetics of this process are well understood for *Pseudomonas aeruginosa*, in which the *ica* gene cluster orchestrates the production of biofilms. Davies et al. 1998 quorum sensing is also likely to be important within biofilms and during biofilm formation.⁷

5 | MATERIALS AND METHODS

Tests: Presumptive tests used for identification of the *Pseudomonas* spp

Brown’s Opacity Tubes: Comparing the turbidity (opacity) of the suspension with the graded turbidities of a series of ten standard tubes (obtainable from, for example, Burroughs Wellcome & Co., London).¹⁴

Plasma: Oxalate or heparin plasma can be used and not citrated plasma because citrate-utilizing bacteria e.g., *Pseudomonas* may cause clotting of the plasma (in tube test). Occasionally, human plasma may contain inhibitory substances which can interfere with coagulase testing can be stored frozen in amounts ready for use.¹² *Pseudomonas* organisms may also produce red colonies on XLD. *Pseudomonas* strains form small green colonies in TCBS agar glucose oxidation positive and glucose fermentation negative,

positive cytochrome oxidase test and pigment production: *P. aeruginosa* produces yellow pyoverdins (fluorescein) and/or pyocyanin (blue aqua pigment). There characteristic grapelike odor of aminoacetophenone as well as growth at 42°C. There are also nonfluorescent *Pseudomonas* species (*P. stutzeri*, *P. alcaligenes*, *P. pseudoalcaligenes*).⁹ Motility was not studied in any of the species of *Pseudomonas*, with the exception of *P. aeruginosa*. Perhaps they are involved in propelling the cell over the surface of solid media (Palleroni et al. 1970; Shinoda and Okamoto 1977). Out of 353 strains, Jessen (1965) found that 19 strains were nonmotile 5 [subsequently acquired] of characteristic of motility. In addition to the polar one *P. Stutzeri* has lateral flagella of shorter wavelength.¹⁰ The study by Okhravi et al. 2000 identified, *Pseudomonas* spp., through detecting bacterial DNA by PCR and by restriction fragment length polymorphism and/or sequencing techniques.

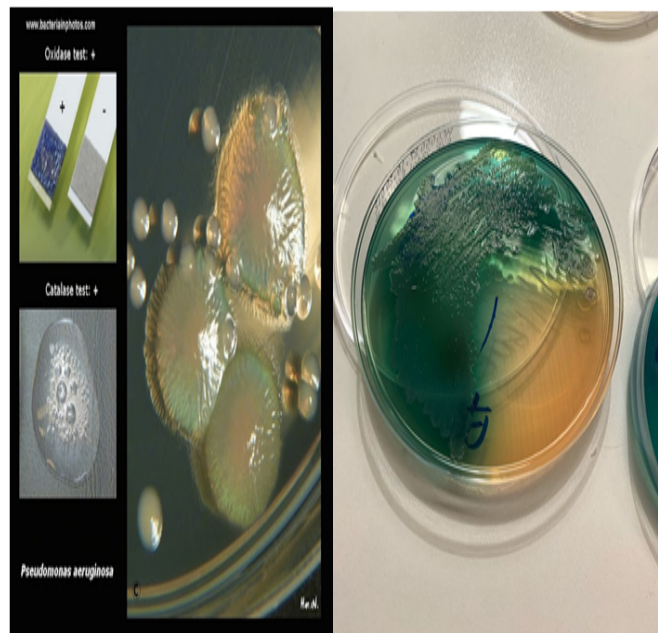
An example of a pigment forming organism is *Pseudomonas aeruginosa* which gives a yellow-green colour in media such as blood agar and MacConkey agar¹². Pyocyanin, a blue phenazine derivative characteristic of *P. aeruginosa*, is diffusible and its production can be enhanced by growth in the ‘King A’ medium (King et al. 1954) recognized as the cause of ‘blue pus’ in wounds, named *Bacillus pyocyaneus* by Gessard the name was changed by Sedillot in 1850 to *Pseudomonas pyocyanea*, and later called *Pseudomonas aeruginosa* by Schroeter in 1872. Chimeric virus-like particles also protected dogs from a lethal challenge with CPV (Langeveld et al. 2001b).

Monoclonal antibodies for outer-membrane proteins have been isolated and characterized by (Hancock et al. 1982). One of the antibodies specific for an antigenic epitope on an outer membrane protein of *P.*

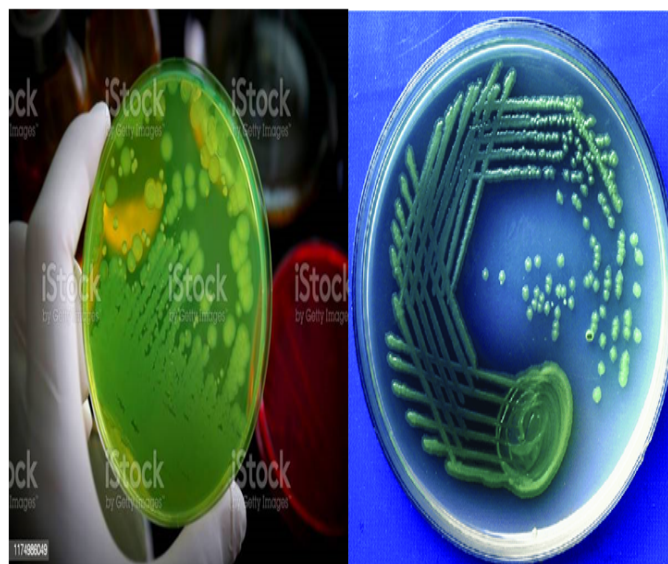
aeruginosa, H2, was recommended as an identification tool for use in clinical laboratories (Mutharia and Hancock 1985) is detected in all 17 serotype strains of the species and, in addition, in other Pseudomonas species that were tested, but not in any species of other genera of aerobic pseudomonads tested.

VACCINE?

A multivalent vaccine based on peptides of CPV, canine distemper virus (CDV), and Pseudomonas aeruginosa could be a commercially viable strategy because they would protect dogs against two important canine diseases and minks against the three major diseases in mink farming.



Pseudomonas answers catalase +oxidase +growth on TSA Pseudomonas on mcConkey agar.



Pseudomonas grown in a cetrimide agar. Pseudomonas in a nutrient agar.

Pseudomonas aeruginosa identified by PNA FISH. PNA FISH is a rapid and highly sensitive and specific fluorescent assay for the detection of Gram-negative pathogens, including P. aeruginosa. The specimen is placed on a slide and fixed. After fixation, a drop of the probe solution is added and hybridized. The slide is washed and read using a fluorescent microscope. The red color signifies hybridization with P. aeruginosa.

	<i>B. cepacia</i>	<i>P.aeruginosa</i>	<i>Burkholderia pseudomallei</i>	<i>P. stutzeri</i>
Associated with cystic fibrosis.	+	+ The mucoid strain results from production of large amounts of alginate, a polysaccharide that surrounds the cell.	["Vietnamese time bomb."] 1960s and 1970s. latitude 20° north and south of the equator (mainly in Thailand and Vietnam).	
Virulence factors toxins		Exotoxin A, endotoxins, proteolytic enzymes, antimicrobial resistance, and production of alginate	Diagnosed only by serological methods.	
	Motile.Green yellow Pigment on blood agar, oxidase +, Glucose OF (open) +, Maltose OF (open)+, Lysine Decarboxylase +Dnase +	Oxidase = + Motility = + Glucose OF (open) = + Gelatin hydrolysis = + Pigment = Red Arginine dihydrolase = + (non fluorescent) Growth at 42°C = + Flagella = + (polar, monotrichous)		
	<i>B. cepacia</i> also produces a yellow pigment MacConkey agar but is motile.	Cetrimide (acetyl trimethyl ammonium bromide) agar is used for the isolation.	Dry, wrinkled colonies that are Tough and adhere to the media as well as smooth Colonies. Positive for cytochrome oxidase, oxidized Glucose and xylose, and grew at 42°C. susceptibility to the polymyxins	Buff to light brown because of the Relatively high concentration of cytochromes. Dry, wrinkled colonies that are Tough and adhere to the media as well as smooth Colonies ⁴
		These organisms can exist in distilled water and underchlorinated water		
		Blue-green pigment on Mueller-hinton agar (pyocyanin pigment)		

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