The study was conducted at the Shree Krishna Hospital located in Karamsad, a tertiary care hospital with a capacity of 550 beds. It is one of the biggest rural based tertiary care hospitals covering a large population of the Anand and Kheda districts of Gujarat state. The hospital provides specialty care in cardiology, gastroenterology, hematology, oncology, nephrology, neurology, infectious diseases and intensive care (medical, surgical, pediatric and neonatal). Apart from this the cardiac unit has its dedicated medical and a surgical intensive care unit.

The hospital also has an advanced Central Diagnostic Laboratory which is well equipped and has been accredited by National Accreditation Board of Laboratories for Testing and Calibration (NABL-15189:2007). The hospital also governs various referral centers in and around the Anand.

This study is prospective observational study. The study was conducted after the approval of the Human Research Ethic's Committee (HREC) of H.M.Patel Centre for medical care and Education and after obtaining the informed consent form from the participants. (Annexure: 1)

Site of study:
Shree Krishna Hospital (tertiary care, teaching, rural hospital) Gokalnagar, Karamsad, Anand District, Gujarat.

Study Duration:
January 2006 to December 2008.

Sample Size:
In the duration of three years, 283 consecutive non repetitive isolates of *Pseudomonas aeruginosa* were obtained from:

1. Various clinical samples such as pus, sputum, urine, blood, endotracheal secretion, cerebrospinal fluid, pleural fluid, peritoneal fluid, ear swabs, conjunctival swabs and
other relevant clinical material submitted to Microbiology Laboratory for Culture and Sensitivity testing from patients of Shree Krishna Hospital, Karamsad.

2. Environmental samples (Sink, respirator, humidifier, transfusion sets, disinfectant solutions, air samples etc.) from the various sites of hospital were periodically collected.

**Inclusion criteria:**

The study includes all the indoor patients of Shree Krishna hospital from whom *Pseudomonas aeruginosa* were isolated from various clinical specimen submitted to Microbiology laboratory for culture and sensitivity testing.

**Exclusion criteria:**

The study excludes the outdoor patients.

**Methodology:**

Various clinical specimens were collected under full aseptic conditions in sterile containers and sent to Microbiology Laboratory of Central Diagnostic Laboratory for culture and sensitivity testing with complete requisition form filled with relevant clinical details of patients. For each patient the following data were collected: age, sex, date of admission, and discharge date, and location. Detailed information on invasive procedures, antibiotic treatment regimen before and after the onset of nosocomial infection, duration of mechanical ventilation, central venous catheterization, insertion sites, urinary catheterization, and surgical interventions were recorded. (Annexure: 3)

Blood sample were collected under aseptic condition and directly inoculated to Brain heart infusion / BacT-Alert (Biomerieux) bottles. (Adult and pediatric bottles were used according to age of patient). (Colour plate nos. 11, 12)
Specimens were received and accepted at Microbiology Laboratory according to acceptance and rejection criteria. Details of patient were entered into computer data base and processed. (Annexure: 2)

Random and surprise microbiological sampling of air from OT's, surface swabs from ICUs/OT's, dressing rooms and Surgical wards and in-use disinfectant from all wards/ICUs and OT's was planned and undertaken as per routine assignment of Microbiology Laboratory for detecting microbial contamination.

After the isolation of *Pseudomonas aeruginosa* from any clinical sample of patient, all the inanimate objects including in-use disinfectants were screened for probable source finding. At the same time all the details regarding patient was obtained and filled in surveillance form. (Annexure: 3)

All the samples were processed in the Microbiology Laboratory according to standard operating procedures (SOPs) laid down by Microbiology Laboratory.

**Day: 1**

**Primary inoculation and Gram staining:**

Pus and wound swabs were inoculated on 5% sheep blood agar and MacConkey agar plates. Sputum and respiratory secretions were inoculated on 5% sheep blood agar, MacConkey agar and chocolate agar plates. Urine and urinary catheter tips were inoculated on CLED agar and MacConkey agar plates. Any catheter tip (Endotracheal catheter tip, tracheostomy catheter tip, central venous catheter tip, Urinary catheter tip) received in the laboratory were injected with sterile peptone water through its lumen with sterile needle and syringe under aseptic precautions and incubated for 2-3hrs in incubator. It was then inoculated to respective culture plates. Body fluids (CSF, peritoneal fluid, pleural fluid, synovial fluid, ascitic fluid, etc.) were inoculated on 5% sheep blood agar, MacConkey agar and chocolate agar plates. After Nov-2007 body
4. Materials and Methods

Fluids were inoculated in to BacT-Alert bottle with sterile needle and syringe, mixed properly and incubated in automated method with BacT-Alert system (Biomerieux) (Colour plate nos. 11, 12). Blood samples were collected in Brain Heart Infusion broth and incubated. After Nov 2007 blood samples were collected in BacT-Alert bottles and incubated in automated method with BacT-Alert system (Biomerieux).

Environmental samples were inoculated and processed further according to standard operating procedures (SOPs) lay down by Microbiology Laboratory.

All the specimens were processed inside the biological safety cabinet (Class II). Smear was made on a clean glass slide for gram staining for provisional identification.

**Incubation:**

Culture plates were incubated aerobically at 35°C-37°C in incubator for 16-18 hours.

Inoculated 5% sheep blood agar and chocolate agar plates were incubated in candle jar at 35°C -37°C aerobically in incubator for 16-18 hours.

Brain heart infusion broth bottles were incubated in a incubator at 35°C-37°C temperature aerobically, subculture was done on 5% sheep blood agar, MacConkey agar and chocolate agar on every alternative day up till seven days.

After Nov. 2007 blood and body fluids were incubated in BacT-Alert system. Subculture was done on 5% sheep blood agar MacConkey agar and chocolate agar when indicated by BacT-Alert system (Positive growth) and simultaneously a smear was made on clean glass slide for gram staining. The inoculated culture plates were incubated at 35°C-37°C in incubator for 16-18 hours.

**Day -2 and -3**

**Culture plates Examination:**

All the inoculated plates were examined for colony morphology and characteristics.

Four to five pure and isolated colonies were inoculated in peptone water broth and
incubated for 3-4 hours at 37°C in incubator for manual identification and antibiotic sensitivity testing and at the same time a smear was made on a clean glass slide for gram staining. If the gram smear showed gram negative bacilli then following biochemical tests were done for manual method identification.

1. Detection of Motility- Semi-solid agar: 279

In semi-solid agar media, motile bacteria ‘swarm’ and give a diffuse spreading growth that is easily recognized by the naked eye.

Method: Bacterial suspension was prepared from colonies and inoculated with a straight wire, made a single stab down the centre of the tube to about half the depth of the medium. Incubated under the conditions favored the motility. Examined the tube and test result was compared with a positive control strain of Pseudomonas aeruginosa ATCC 27853

Non-motile bacteria generally give growths that are confined to the stab-line have sharply defined margins and leave the surrounding medium clearly transparent. Motile bacteria typically give diffuse, hazy growths that spread throughout the medium rendering it slightly opaque.

2. Catalase test: 279

This demonstrate the presence of Catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide.

Method: In a sterile glass tube 1 ml 3% hydrogen peroxide was taken. Two or three isolated colonies were picked up from MacConkey agar with a sterile stick and were put in to the hydrogen peroxide containing tube. Immediate bubble formation indicated as Catalase positive. (Colour Plate no. 6)

The test result was compared with a positive control strain of Pseudomonas aeruginosa ATCC 27853.
3. Oxidase test:

This test depends on the presence in bacteria of certain oxidases that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye-tetramethyl-p-phenylene-diamine. The dye is reduced to a deep purple colour.

**Method:** Dry filter paper method

Ready to use disc of filter paper from Hi-media Laboratory were used to perform above test. The colonies to be tested were picked with a plastic or wooden stick and smeared over the moist area of the Oxidase disc. A positive reaction was indicated by an intense deep-purple hue, appeared within 5-10 seconds, a delayed positive reaction by coloration in 10-60 seconds, and a negative reaction by absence of coloration or by coloration later than 60 seconds. (Colour Plate no. 6)

The test result was compared with a positive control strain of *Pseudomonas aeruginosa* ATCC 27853.

4. Urease test:

Bacteria, particularly those growing naturally in an environment exposed to urine, may decompose urea by means of the enzyme urease:

\[
\text{NH}_2\text{CO.NH}_2 + \text{H}_2\text{O} = 2\text{NH}_3 + \text{CO}_2
\]

The occurrence of this enzyme can be tested for by growing the organism in the presence of urea and testing for alkali (NH₃) production by means of suitable pH indicator.

**Method:** Entire slop surface of Urease medium was heavily inoculated and incubated at 37°C. Examination was done after 4 hr. and after overnight incubation, and also further incubated for two days more before being reported negative. Urease positive cultures changed the colour of the indicator to purple-pink. (Colour Plate no. 6)

The test result was compared with a positive control strain of *Pseudomonas aeruginosa* ATCC 27853.
5. **Triple Sugar Iron agar (TSI agar) test:**

This medium was originally designed as a multi-test medium. It provides a low degree of sensitivity for H₂S production. The medium is now used principally as a standard test for H₂S.

**Method:** Slop surface was inoculated with heavy inoculum and stabbed in to the butt. Incubated aerobically at 37°C for 24 hrs. Result was interpreted. (Colour Plate no. 6)

<table>
<thead>
<tr>
<th>Slant/but</th>
<th>Color</th>
<th>Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline/acid</td>
<td>Red/yellow</td>
<td>Glucose only fermented; peptone utilized</td>
</tr>
<tr>
<td>Acid/acid</td>
<td>Yellow/yellow</td>
<td>Glucose fermented, lactose and/or sucrose fermented</td>
</tr>
<tr>
<td>Alk/alk</td>
<td>Red/red</td>
<td>No fermentation of any of the sugar; peptone utilized</td>
</tr>
</tbody>
</table>

The test result was compared with a positive control strain of *Pseudomonas aeruginosa* ATCC 27853.

6. **Indole test:**

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane to indole, which accumulates in the medium. Indole is then tested for by a colorimetric reaction with *p*-dimethyl-aminobenzaldehyde.

**Method:** Medium was inoculated and incubated for 48 hrs at 37°C may be required for optimum accumulation of Indole. Then 0.5 ml Kovac’s reagent was added. A red colour at the top of the test tube indicated positive test. (Colour Plate no. 6)

The test result was compared with a positive control strain of *Pseudomonas aeruginosa* ATCC 27853.

7. **Methyl Red test:**

The methyl red test is employed to detect the production of sufficient acid during the fermentation of glucose and the maintenance of condition such that the pH of an old
4. Materials and Methods

A culture is sustained below a value of about 4.5, as shown by a change in the color of the methyl red indicator which is added at the end of the period of incubation.

**Method:** The liquid medium Glucose Phosphate broth was inoculated lightly with a young culture and incubated at 37°C for 48 hrs then added 5 drops of methyl red reagent. Mixed and noted the result. Positive test was bright red and negative test was yellow. In case of equivocal result, test was repeated and incubated further for 5 days.

(Colour Plate no. 6)

The test result was compared with a positive control strain of *Pseudomonas aeruginosa* ATCC 27853.

**8. Voges-Proskauer (acetoin production):**

Many bacteria ferment carbohydrates with the production of acetyl methyl carbinol (CH$_3$.CO.CH$_2$.CHOH.CH$_3$) or its reduction product 2, 3 butylene glycol (CH$_3$.CHOH.CH$_2$.CHOH.CH$_3$). The substances can be tested for by a colorimetric reaction between diacetyl (CH$_3$.CO.CO.CH$_3$- formed during the test by oxidation of acetyl methyl carbinol or 2, 3 butylene glycol) and a guanidino group under alkaline condition. This test usually done in conjugation with the methyl red test since the production of acetyl methyl carbinol or butylene glycol usually results in insufficient acid accumulating during fermentation to give a methyl red positive reaction. An organism of the enterobacterial group is usually either methyl-red-positive and Voges-Proskauer-negative or methyl-red negative and Voges-Proskauer-positive.

**Medium:** Glucose phosphate broth

**Method:** The liquid medium Glucose Phosphate broth was inoculated lightly with a young culture and incubated at 37°C for 48 hrs. 0.5 ml of O’Meara reagent (40 gm potassium hydroxide and 0.3 g creatine in 100 ml distilled water) was added. Tubes were placed in a 37°C water bath for 4hrs. Tubes were aerated by shaking at intervals. A
positive reaction was denoted by a development of an eosin-pink color, usually in 2-5 minutes. (Colour Plate no. 6)

The test result was compared with a positive control strain of *Pseudomonas aeruginosa* ATCC 27853.

9. **Citrate Utilization test:**

This is a test for the ability of an organism to utilize citrate as the sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen. Koser’s liquid citrate medium or Simmons’ citrate agar may be used.

**Medium:** Simmons’ citrate medium

**Method:** Inoculated medium from saline suspension of the organism and incubated at 37°C for 96 hrs. The result was noted. Positive reaction-blue colour and Negative reaction-original green color. (Colour Plate no. 6)

The test result was compared with a positive control strain of *Pseudomonas aeruginosa* ATCC 27853.

**Antibiotic Susceptibility Testing:**

Antimicrobial susceptibility of all the isolates was performed by Kirby-Bauer disc diffusion method as per CLSI guidelines. The following antibiotics (Hi-Media) were tested. (Colour plate no. 7)

- Ceftazidime (30 µg),
- Gentamicin (10 µg),
- Tobramycin (10 µg),
- Piperacillin (100 µg),
- Amikacin (30 µg),
- Aztreonam (30 µg),
- Cefipime (30 µg),
4. Materials and Methods

- Ciprofloxacin (5 µg),
- Levofloxacin (5 µg),
- Imipenem (10 µg),
- Meropenem (10 µg),
- Piperacillin/Tazobactam (100/10 µg),
- Ticarcillin (75 µg),
- Ofloxacin (5 µg),
- Norfloxacin (10 µg)

**mini AP1 system (Biomerieux):**

The mini AP1 has identification and sensitivity strips 1D GN32 and ATB PSE 5 respectively. (Colour plate nos.9, 10)

**ID 32 GN (Automatic identification system for Gram-negative rods):**

The ID 32 GN is a standardized system for the automatic identification of Gram-negative rods, which uses 32 miniaturized assimilation tests and a database. The complete list of those organisms that it is possible to identify with this system can be found in the percentage table. This strip is designed to be used exclusively with the ATB™ Expression™ or mini API® instruments. (Colour Plate no.:9)

**Principle:** The ID 32 GN strip consists of 32 cupules, each containing a dehydrated carbohydrate substrate. A semi-solid, minimal medium is inoculated with a suspension of the organism to be tested. After 24-48 hrs of incubation, growth in each cupule is detected by an automatic reader and identification is obtained using the ATB Expression or mini API instruments.

**Composition of the Strip:**

The composition of the ID 32 GN strip is given below in the list of tests.
### 4. Materials and Methods

<table>
<thead>
<tr>
<th>CUPULES</th>
<th>TESTS</th>
<th>SUBSTRATES</th>
<th>QTY (mg/cup)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>RHA</td>
<td>L-RHAMnose</td>
<td>0.68</td>
</tr>
<tr>
<td>1.1</td>
<td>NAG</td>
<td>N-Acetyl-Glucosamine</td>
<td>0.68</td>
</tr>
<tr>
<td>1.2</td>
<td>RIB</td>
<td>D-RiBose</td>
<td>0.70</td>
</tr>
<tr>
<td>1.3</td>
<td>INO</td>
<td>INOsitol</td>
<td>0.70</td>
</tr>
<tr>
<td>1.4</td>
<td>SAC</td>
<td>D-SACcharose (Sucrose)</td>
<td>0.66</td>
</tr>
<tr>
<td>1.5</td>
<td>MAL</td>
<td>D-MALose</td>
<td>0.70</td>
</tr>
<tr>
<td>1.6</td>
<td>ITA</td>
<td>ITAconic acid</td>
<td>0.23</td>
</tr>
<tr>
<td>1.7</td>
<td>SUB</td>
<td>SUBeric acid</td>
<td>0.35</td>
</tr>
<tr>
<td>1.8</td>
<td>MNT</td>
<td>Sodium MaloNaTe</td>
<td>1.20</td>
</tr>
<tr>
<td>1.9</td>
<td>ACE</td>
<td>Sodium ACETate</td>
<td>0.55</td>
</tr>
<tr>
<td>1.A</td>
<td>LAT</td>
<td>LAcTic acid</td>
<td>0.32</td>
</tr>
<tr>
<td>1.B</td>
<td>ALA</td>
<td>L-ALAnine</td>
<td>0.68</td>
</tr>
<tr>
<td>1.C</td>
<td>SKG</td>
<td>Potassium 5-KetoGluconate</td>
<td>0.90</td>
</tr>
<tr>
<td>1.D</td>
<td>GLYG</td>
<td>GLYcoGen</td>
<td>0.64</td>
</tr>
<tr>
<td>1.E</td>
<td>mOBE</td>
<td>3-hydrOxyBEnzoic acid</td>
<td>0.23</td>
</tr>
<tr>
<td>1.F</td>
<td>SER</td>
<td>L-SERine</td>
<td>0.80</td>
</tr>
<tr>
<td>0.0</td>
<td>MAN</td>
<td>D-MANitol</td>
<td>0.68</td>
</tr>
<tr>
<td>0.1</td>
<td>GLU</td>
<td>D-GLUcose</td>
<td>0.78</td>
</tr>
<tr>
<td>0.2</td>
<td>SAL</td>
<td>SALicin</td>
<td>0.52</td>
</tr>
<tr>
<td>0.3</td>
<td>MEL</td>
<td>D-MELibiose</td>
<td>0.66</td>
</tr>
<tr>
<td>0.4</td>
<td>FUC</td>
<td>L-FUCose</td>
<td>0.64</td>
</tr>
<tr>
<td>0.5</td>
<td>SOR</td>
<td>D-SORbitol</td>
<td>0.68</td>
</tr>
<tr>
<td>0.6</td>
<td>ARA</td>
<td>1-ARAbinose</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Medium: API AUX medium was used.

**Preparation of the inoculum:**

An ampule of API Nacl 0.85% medium opened and one or several identical colonies from the young culture were added and bacterial suspension was prepared. Turbidity of the suspension was adjusted equivalent to 0.5 McFarland and measured with the ATB Densitometer. An ampule of API AUX medium was opened and 200µl of the bacterial suspension was added. (Colour plate no. 8)

**Inoculation of the Strip:**

An ampule was homogenized and inoculated 135µl of the suspension in to each cupule of the strip using ATB electronic pipette. Lid was placed on the strip and incubated at 29°C ± 2°C for 24 hrs.

**Reading and Interpretation:**

Reading and interpretation was performed automatically by mini API instrument using the database.

The isolates were identified as *Pseudomonas aeruginosa.*

<table>
<thead>
<tr>
<th>0.7</th>
<th>PROP</th>
<th>PROPionic acid</th>
<th>0.29</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>CAP</td>
<td>CAPric acid</td>
<td>0.11</td>
</tr>
<tr>
<td>0.9</td>
<td>VALT</td>
<td>VALeric acid</td>
<td>0.25</td>
</tr>
<tr>
<td>0.A</td>
<td>CIT</td>
<td>Trisodium CITrate</td>
<td>0.57</td>
</tr>
<tr>
<td>0.B</td>
<td>HIS</td>
<td>L-HIStidine</td>
<td>0.80</td>
</tr>
<tr>
<td>0.C</td>
<td>2KG</td>
<td>Potassium 2-KetoGluconate</td>
<td>0.98</td>
</tr>
<tr>
<td>0.D</td>
<td>3OBU</td>
<td>3-hydROxyBUtyric acid</td>
<td>0.30</td>
</tr>
<tr>
<td>0.E</td>
<td>pOBE</td>
<td>4-hydROxyBEnzoic acid</td>
<td>0.23</td>
</tr>
<tr>
<td>0.F</td>
<td>PRO</td>
<td>L-PROline</td>
<td>0.52</td>
</tr>
</tbody>
</table>
**ATB™ PSE 5 (Susceptibility test for Pseudomonas and other nonfermenters):**

The ATB PSE 5 strip enables the determination of the susceptibility of *Pseudomonas* and other nonfermenting Gram-negative rods to antibiotics in a semi-solid medium under conditions similar to the reference methods for agar dilution or micro-dilution.

**Principle:** The ATB PSE5 strip consists of 16 pairs of cupules. The first pair does not contain any antibiotics and is used as a positive growth control. The next 14 pairs contain antibiotics at one single or two concentrations (c and C). The last pair is empty. A suspension is prepared with the bacterium to be tested then transferred into the strip. After incubation, reading of the growth in the cupules is performed automatically by the mini API. The result obtained classifies the strain as Sensitive, Intermediate or Resistant.

**Preparation of the inoculum:**

A bacterial suspension was prepared with a turbidity equivalent to 0.5 McFarland by using Densitometer.

**Inoculation of the Strip:** ATB medium was homogenized with electronic micropipette avoiding the formation of bubbles. Strip was inoculated with by dispensing 135 µl of ATB medium in to each cupule using the ATB Electronic Pipette (approximately 2×10^5 organisms/ml or 3×10^4 organisms/cupule)

**Reading and interpretation:** Reading was taken on mini API instrument. Interpretation was done automatically by mini API instrument following CLSI guideline.

Following drugs were tested for antimicrobial susceptibility: ampi-sulbactam, ticarcillin, ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, imipenem, mero-penem, amikacin, gentamicin, tobramycin, ciprofloxacin, and colistin.

After Nov 2007, antibiotic susceptibility of all the isolates was also done by the
automated system (mini API) by sensitivity strips ATB PSE 5 (Biomerieux, France).

**Metallo-β-lactamase Screening:**
A total of 283 consecutive clinical isolates of *P. aeruginosa* were collected and confirmed. All the confirmed *P. aeruginosa* isolates were subjected to Kirby-Bauer disc diffusion method as per CLSI guidelines. Imipenem resistance by disc diffusion method was found in 38 out of 283 isolates of *Pseudomonas aeruginosa*. Of 38 isolates, 35 isolates were tested for metallo-β-lactamase producer.

**Imipenem (IMP)-EDTA combined disc test:**
To identify metallo-β-lactamase (MBL) production in these isolates, we used IPM-EDTA-disk synergy test developed by Yong D *et al.* To make 0.5 M EDTA solution 186.1 g of disodium EDTA was dissolved in 1000 mL of distilled water and pH was adjusted to 8.0 by using NaOH. The mixture was then sterilised by autoclaving. EDTA imipenem disks were prepared by adding EDTA solution to 10-μg-imipenem disks to obtain a concentration of 750 μg. The disks were dried immediately in an incubator and stored at 4°C or at −20°C in an air tight vial without desiccant. Test strains were adjusted to the McFarland 0.5 standard and were inoculated to Mueller Hinton agar. A 10-μg-imipenem disk and an imipenem plus 750 μg EDTA were placed on Mueller Hinton agar. Another disk containing only 750 μg EDTA was also placed as a control. After overnight incubation, the established zone diameter difference of ≥ 7 mm between imipenem disk and imipenem plus EDTA was interpreted as EDTA synergy positive. (Colour plate no:13)

ATCC 27853 strain of *Pseudomonas aeruginosa* was used as a control strain when ever required.
**Definitive Identification of Pseudomonas aeruginosa:**

Identification based on all of the following:

1. Gram-negative rod
2. Oxidase-positive
3. Typical smell (fruity grape-like odor)
4. Recognizable colony morphology

(a) On blood or chocolate agar appears as large colonies with metallic sheen, mucoid, rough, or pigmented (pyocyanin) and often β-hemolytic.

(b) On MacConkey, appear as lactose-negative with green pigmentation or metallic sheen.

More than 95% of *Pseudomonas aeruginosa* strains recovered from clinical specimens can be identified by observing the presence of above primary characteristics. Most strains produce pyocyanin, a water-soluble green phenazine pigment that imparts a greenish color to the culture medium. In fact, observing the presence of pyocyanin may be the only characteristic required to identify *Pseudomonas aeruginosa*, because no other nonfermenters synthesizes this pigment. Some mucoid strains of *Pseudomonas aeruginosa* from patients with CF may not produce pigment and, therefore, may be misidentified if pigment production is the only criterion used for identification of these aberrant strains. Detecting the grapelike odor is also helpful clue when examining the growth on agar palates. The colonies are large, may be mucoid or dry, and often spread.

A few strains of *Pseudomonas aeruginosa* may produce pigment with other colors—pyorubin (red), pyomelanin (brown to black), and pyoverdin (yellow). Muller-Hinton agar is suitable medium for demonstrating fluorescence. Variants producing mucoid or dwarf colonies with atypical biochemical reaction may also be encountered, occasionally making identification difficult.
In summary, most strains of *Pseudomonas aeruginosa* can be identified easily by observing the typical large colonies, with a blue-green discoloration on primary isolation media, and further confirmed by detecting a typical grapelike odor. Demonstration of fluorescein pigment and cytochrome oxidase activity helps to confirm the final identification, and additional tests are usually not required.