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# Clinical distribution and antibiotic resistance of Pseudomonas species*

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**Objective** The aim of this study was to determine the isolation properties of *Pseudomonas aeruginosa* and other species, distribution rates in clinics and resistance to antibiotics in Akdeniz University Hospital.

**Method** Pseudomonas bacteria were isolated and identified by conventional methods. The antibiotic resistance rates were detected by minimal inhibitory concentration (MIC). The clinical and specimen distribution properties of Pseudomonas were evaluated based on their resistance.

**Results** Pseudomonas bacteria were the fourth common in all isolates. Tracheal aspirates, sputum and wound pus were important sources for *P.aeruginosa* isolation in intensive and nonintensive care units of surgery wards (SW-ICU, SW-nonICU) ($p<0.05$). On the basis of $MIC_{90}$ criteria, the resistance ratios of the isolates to ceftriaxone (CRO), cefotaxime (CTX), ceftazidime (CAZ), imipenem (IP), ofloxacin (OFX), ciprofloxacin (CIP) were 8.4%, 15.0%, 13.3%, 0.0%, 11.6% and 8.3% respectively. The table illustrates the statistical properties of the resistance of isolates which show significant relations between clinics and specimens.

| | | CRO | CTX | CAZ | OFX | CIP |
|---|---|---|---|---|---|---|
| Tracheal asp. | SW-ICU | p<0.001 | p<0.001 | p>0.05 | p<0.001 | p<0.001 |
| Wound pus | SW-ICU | p<0.001 | p<0.001 | p<0.001 | p<0.001 | p<0.001 |
| Sputum | SW-nonICU | p<0.001 | p<0.001 | p<0.05 | p>0.05 | p>0.05 |
| Wound pus | SW-nonICU | p<0.001 | p<0.001 | p>0.05 | p<0.001 | p<0.001 |

No resistance to imipenem was observed.

**Conclusion** These data show that it would be useful to apply more effective antibiotic control programmes in surgery wards.

**Key words** Pseudomonas species, resistance

## Introduction

*Pseudomonas aeruginosa* remains an important cause of hospital-acquired infections particularly among immunosupressed patients (and those carrying prosthetic devices) in intensive care units (ICU). The divercity of clinics and the regional variations in antibiotic protocols result in the different resistance profiles (1-3).

In this study, isolation, distribution and susceptibility properties of *P.aeruginosa* and other species were determined in Akdeniz University Hospital.

## Material and Method

Akdeniz University Hospital has 400 beds and receives about 7500 admissions per year. On each floor, there are two or three clinics and each room contains six beds. There is an infection control committee in the university hospital. Only patients in internal wards-ICU are not moved, but those in all other wards may be moved between wards and floors.

Only 7438 samples were evaluated in the first six-month period of 1995. Giemsa and gram stained smears of sputum and tracheal aspirates were evaluated for quality scores. The Bact/Alert system was used for blood cultures. Urine samples were from immunesuppressive patients were evaluated according to prominent colonies and clinical correlations.

*Identification*

The isolates were identified by conventional methods. Strains were identified as Pseudomonas on the basis of colony morphology, gram stain, oxidase reaction, development of pyocyanin pigments and urease enzyme, nitrate reduction and production of gases, gelatin hydrolysis, uses of citrate and malonate as a carbon source, growing capability at 5°C and 42°C. Glucose, lactose, maltose, mannitol, sucrose and xylose were used to investigate the acide production in sugars.

*Susceptibility tests*

The susceptibility testing of *P.aeruginosa* was performed with standardized broth MIC tests according to NCCLS suggestions.

*Statistical analysis*

The SPSS for Windows Ver 6.0 was used for statistical analysis. Multiple-line khi-square analysis was done (Table VIII).

## Results

The rate of pathogenic bacteria was 32.72% in 7438 samples. The nonfermentative gram (-) bacilli were the fourth common bacteria in all isolates

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(Table I). Pseudomonas sp were the most common bacteria out of all nonfermentative gram (-) rods (Table II). *P.aeruginosa* was the commonest bacteria out of all Pseudomonas species (Table III). Pseudomonas sp. were the most frequently isolated from ICU ($p<0.05$, Table IV). Pseudomonas sp. were the most frequently isolated from surgical wards ($p<0.05$, Table V). There is no difference in isolation rate between SW-ICU and SW-nonICU ($p>0.05$, Table VI). There was a higher isolation rate in IW-ICU than IW-nonICU ($p<0.05$, Table VII). Isolation rates of Pseudomonas sp from tracheal aspirate, sputum, pus and catheter samples were higher than the other samples.

Table I. Types of bacteria isolates from samples

| Bacteria | Isolation | | Rate in total |
|---|---|---|---|
| | n | % | (n=7438) % |
| Staphylococcus sp. | 681 | 27.98 | 9.16 |
| Streptococcus sp. | 588 | 24.16 | 7.91 |
| Enterobacteriaceae | 586 | 24.08 | 7.88 |
| Nonfermentative Gr(-) Bacilli | 286 | 11.74 | 3.84 |
| Yeasts | 190 | 7.80 | 2.55 |
| Gr(+) Bacilli | 60 | 2.47 | 0.80 |
| Others* | 43 | 1.77 | 0.58 |
| Total | 2434 | 100.00 | 32.72 |

*:Neisseria sp, Haemophilus sp, Aeromonas sp

Table II. Distribution of nonfermentative gram (-) rods

| Nonfermentative gram-negative rods | Isolation (n) | Distribution rate (%) |
|---|---|---|
| Pseudomonas sp. | 192 | 67.13 |
| Acinetobacter sp. | 62 | 21.68 |
| Alcaligenes sp. | 8 | 2.80 |
| Xanthomonas maltophilia | 6 | 2.10 |
| Ochrobactrum anthropi | 5 | 1.75 |
| Oligella urethralis | 1 | 0.35 |
| Unidentified | 12 | 4.19 |
| Total | 286 | 100.00 |

Table III. Distribution of Pseudomonas by species

| Pseudomonas sp. | Isolation (n) | Distribution rate (%) |
|---|---|---|
| *Pseudomonas aeruginosa* | 176 | 91.67 |
| *Pseudomonas cepacia* | 10 | 5.21 |
| *Pseudomonas putida* | 2 | 1.04 |
| *Pseudomonas stutzeri* | 2 | 1.04 |
| *Pseudomonas vesicularis* | 1 | 0.52 |
| *Pseudomonas mendocina* | 1 | 0.52 |
| Total | 192 | 100.00 |

Table IV. Pseudomonas species isolated from ICU and non-ICU

| | Total sample | Pseudomonas sp. | % |
|---|---|---|---|
| Intensive Care Units (ICU) | 1528 | 67 | 4.38 |
| Non-Intensive Care units (nonICU) | 5910 | 125 | 2.11 |

$p<0.05$

Table V. Pseudomonas species isolated from surgical wards and internal wards

| | Total sample | Pseudomonas sp. | % |
|---|---|---|---|
| Surgery Wards (SW) | 2397 | 112 | 4.67 |
| Internal Wards (IW) | 5041 | 80 | 1.58 |

$p<0.05$

Table VI. Pseudomonas species isolated from SW-ICU and SW-nonICU

| | Total sample | Pseudomonas sp. | % |
|---|---|---|---|
| Surgery Wards-Intensive Care Unit (SW-ICU) | 1023 | 51 | 4.98 |
| Surgery Wards-Non Intensive Care Unit (SW-nonICU) | 1374 | 61 | 4.43 |

$p>0.05$

Table VII. Pseudomonas species isolated from IW-ICU and IW-nonICU

| | Total sample | Pseudomonas sp. | % |
|---|---|---|---|
| Internal Wards-Intensive Care Units (IW-ICU) | 505 | 16 | 3.16 |
| Internal Wards-Non Intensive Care Units (IW-nonICU) | 4536 | 64 | 1.41 |

$p<0.05$

Table VIII. Distribution of isolation frequency by sample

| Sample | Amount (N) | Pseudomonas sp. (n) | (%) | p |
|---|---|---|---|---|
| **Tracheal aspiration** | **97** | **19** | **19.59** | **<0.05** |
| **Sputum** | **427** | **48** | **11.27** | **<0.05** |
| **Wound pus** | **842** | **63** | **7.48** | **<0.05** |
| **Catheter** | **53** | **2** | **3.77** | **<0.05** |
| Urine | 2331 | 46 | 1.97 | >0.05 |
| Blood | 1210 | 7 | 0.58 | >0.05 |
| Throat* | 1186 | 5 | 0.42 | >0.05 |
| Periton | 321 | 1 | 0.31 | >0.05 |
| Stool* | 635 | 1 | 0.16 | >0.05 |
| Others | 336 | 0 | 0.00 | >0.05 |
| Total | 7438 | 192 | 2.58 | - |

*: Precence of immunosuppressive conditions

It was investigated whether or not there is any relationship between sample and unit on frequency of Pseudomonas sp isolates. The isolation rates of Pseudomonas were higher from sputum and pus in SW, tracheal aspirates and pus

in SW-ICU and tracheal aspirates in IW-ICU (Table IX).

Table IX. Distribution of Pseudomonas sp. in various units

| | | IW | SW | IW-nonICU | SW-nonICU |
|---|---|---|---|---|---|
| | Sample (n) | | | 60 | 27 |
| Tracheal | Pseudomonas sp. | - | - | 2 | 17 |
| Aspiration | % | | | 3.33 | 62.96 |
| | p | | | p>0.05 | **p<0.001** |
| | Sample (n) | 280 | 48 | 22 | 77 |
| Sputum | Pseudomonas sp. | 20 | 8 | 5 | 15 |
| | % | 7.14 | 16.66 | 22.72 | 19.48 |
| | P | p>0.05 | **p<0.001** | p>0.05 | p>0.05 |
| | Sample (n) | 111 | 642 | 39 | 50 |
| Wound pus | Pseudomonas sp. | 13 | 27 | 6 | 17 |
| | % | 11.71 | 4.2 | 15.38 | 34 |
| | p | p>0.05 | **p<0.001** | p>0.05 | **p<0.05** |
| | Sample (n) | | | | 28 |
| Catheter | Pseudomonas sp. | - | - | - | 2 |
| | % | | | | 7.14 |
| | p | | | | p>0.05 |

Table X. $MIC_{90}$ values and resistance percentages of *P.aeruginosa*

| Antibiotic | $MIC_{90}$ (μg/ml) | Resistant rate(%) |
|---|---|---|
| Ceftriaxone (CRO) | 32 | 8.4 |
| Cefotaxime (CTX) | 64 | 15.0 |
| Ceftazidime (CAZ) | 32 | 13.3 |
| Imipenem (IP) | 1 | 0.0 |
| Ofloxacin (OFX) | 16 | 11.6 |
| Ciprofloxacin (CIP) | 2 | 8.3 |

No resistance to imipenem was found. The highest resistance was to cefotaxime and ceftazidime (Table X).

Table XI shows cross-table statistical analysis of antibiotics and isolation sites which have significant p values in Table IX.

Table XI. Statistical properties of isolate resistance

| | | CRO | CTX | CAZ | IP | OFX | CIP |
|---|---|---|---|---|---|---|---|
| Tracheal aspiration (N=27; n=17) | SW-ICU | p<0.001 | p<0.001 | p>0.05 | NT | p<0.001 | p<0.001 |
| Wound pus (N=50; n=17) | SW-ICU | p<0.001 | p<0.001 | p<0.001 | NT | p<0.001 | p<0.001 |
| Sputum (N=48; n=8) | SW-nonICU | p<0.001 | p<0.001 | p<0.05 | NT | p>0.05 | p>0.05 |
| Wound pus (N=642; n=27) | SW-nonICU | p<0.001 | p<0.001 | p>0.05 | NT | p<0.001 | p<0.001 |
| Resistance rates (%) | | 8.4 | 15.0 | 13.3 | 0.0 | 11.6 | 8.3 |

NT: Not tested (resistance rate= 0.0)

N: Frequency of isolation site n: Frequency of isolated P.aeruginosa

## Discussion

*Pseudomonas aeruginosa* is a major cause of nosocomial infection. Despite advances in sanitation facilities and the introduction of wide variety of antimicrobial agents with antipseudomonal activities, life-treatening infections caused by *P.aeruginosa* continue to be hospital infections. The distribution of isolates is significantly effected by the type of hospital (general, teaching or specialized). Isolation due to nosocomial infection was 3-16% in multi-center studies. *P.aeruginosa* was the most common pathogen in nosocomial infections. It is the leading cause of nosocomial respiratory tract infections (3-9). Our findings support this results (Table I-III,VIII).

Nonfermentative gram negative rods are environmental organisms. They are infrequently found as a part of the human microflora of healthy individuals. *P.aeruginosa* is more frequently isolated than other nonfermentative gram negative rods. It carries importance as a nosocomial pathogen; the isolation rate was 68.4% (10).

*P.cepacia* was the second common pathogen after *P.aeruginosa.* It was established in immunosupressed patients with respiratory tract infections and it is a contaminant bacteria in pharmaceutical industries. Its

isolation rate was 3.1-4% (10-11). Our result (5.21%) was consistant with this percentage.

*P.putida* was the agent of catheter-related bacteremia, isolated from sputum, pus, joint and peritoneal fluid and urine related infections. The isolation rate was 1.1% in Suzuki's study (10). This was similar to ours (1.04%).

*P.stutzeri* is an unusual cause of human infection and it was reported as a pathogen in 1895. It was isolated from urine, blood, respiratory tract of intubated patients and wounds (11-12). In the report of Noble et al, the isolation rate of *P.stutzeri* was 2.0% (12). This percentage was higher than our result.

*P.vesicularis* may frequently colonize aqueous hospital environments. *P.vesicularis* bacteremia, genital and wound infections were reported (13-15). Our *P.vesicularis* bacteria were isolated from wound.

*P.mendocina* is an industrial bacteria used for alginate synthesis. It was reported as an agent of endocarditis (16). *P.mendocina* was isolated from peritoneal dialisate in our study.

Pseudomonas species other than aeruginosa are less virulant. Immunsuppression, contaminated disinfectant solutions and transfusion products and use of auxilliary medical equipment are predisposing factors leading to low virulent bacteria becoming an agent of infection. Intensive care patients create an environment for infection because of the debilitating effect of a prolonged hospitalisation and the application of medical equipment (airways, catheters etc) (2,3,17). Isolation rate of Pseudomonas sp. was significant for ICU in our study ($p<0.05$, Table IV).

Surgical wards are the units where patients are hospitalized for a long period of time and medical equipment (catheter, sound, etc) is used. This equipment predisposes opportunistic infections if there isn't effective isolation (1). Multidrug resistant *P.aeruginosa* has frequently been reported as the cause of nosocomial outbreaks of infection in surgery wards (9). In our hospital, Pseudomonas isolations were higher in surgical wards than medical wards ($p<0.05$). But there was no significant difference in Pseudomonas isolation rates between ICU and non ICU surgical wards where effective isolation couldn't be enforced. Pseudomonas isolation is generally carried out in IW-ICU on patients suffering from chronic and malignant diseases who are using immunosuppressed drugs and/or medical prosthetis devices. The rate of Pseudomonas infections in IW-ICU was higher than in IW-nonICU in our hospital ($p<0.05$).

In this study, tracheal aspirate is an important source of Pseudomonas sp only in internal wards of ICU ($p<0.05$) because Pseudomonas colonies in the airway and infection are able to spread in IW-ICU (18). Specimens are generally taken in aseptic conditions in surgical wards, but epithelial and mucosal erosions lead to Pseudomonas colonisation. In a report from France, *P.aeruginosa* was the commonest bacteria of the respiratory tract in SW-ICU (17). In our report, isolation from tracheal aspirates in surgical wards ICU and sputum in SW-nonICU have a significant value (62.96%, $p<0.001$; 16.66%, $p<0.05$). Isolation from pus in SW and ICU, and non-ICU were important (4.2%, $p<0.001$; 34%, $p<0.05$). Also isolations from pus were important in SW and ICU (4.2%, $p<0.001$; 34%, $p<0.05$). The impairment of the skin, the presence of surgical instruments, for example catheter, the contaminated washing out solutions used by the staff and hospitalization for a long period of time are the predisposing factors for the Pseudomonas sp infections (19). 13-22.1% *P.aeruginosa* isolation rate was reported from pus (20,21).

Immunsuppressed patients are hospitalized for a long period and they generally use high doses of antibiotic. It is necessary to take the throat culture and detect resistance before admittance to hospital in order to detect carriers.

CRO and CTX are the commonest 3$^{th}$ generation antibiotics in ICU protocols. CAZ has a key role in resistance detection in ICU. But ICU antibiotic therapy protocols are different in almost all countries and there are regional differanciences (22). $MIC_{90}$ values were reported as 8 μg/ml and ≥38 μg/ml by Kessler et al and Verbist et al respectivetely (23,24). These rates corresponded to our study (32 μg/ml). Resistance to CAZ was reported as 4-18 in ICU (24-27). That is consistant with our result(13.3%). Resistance to CRO and CTX are significant in our study (8.4-15.0%). There was cross resistance between these two antibiotics because of the similarity of molecular structure (22). Previous studies suggest that the selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains. The resistance to quinolone group antibiotics of Pseudomonas is variable in different centers. Resistance to CIP in ICU was reported as 8-31% (1, 26, 29, 30). Quinolone resistance in our study is the same as these reports (Table X). Nowadays, quinolones are the recommended antibiotics for respiratory tract infections. It is unusual to find resistance to quinolone in isolates from sputum (Table XI). This resistance should be discussed according to the source of the material and clinics because of prologed and overused antibiotic usage. In report from Sweden, quinolone resistance was always crossed to resistance to either or both of the β-lactams and the aminoglycosides (22,30).

Minimizing and controlling antibiotic use in ICU patients should help reduce the risk of antimicrobial resistance in Pseudomonas bacteria. The extensive use of broad-spectrum antibiotics in the hospital can be probably responsible for the emergence and selection of resistant strain.

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