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Providencia spp in Nosocomial Urinary Infections

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Abstract: This study was carried out in the Ankara Rehabilitation Centre over an eight month period. A total of 428 urine samples was collected from inpatients with long-term catheterization for diagnosis of bacteriuria. Urinary tract infection was detected in three hundreds and thirty-one samples. *Escherichia coli* was found to be the highest causative agent. Moreover in forty of these samples *Providencia* spp were determined as the fifth highest causative agent. This *Providencia* spp finding is important as it is the highest recorded in Turkey. Although six out of 40 isolates were during preservation, the remaining thirty-four isolates were further investigated to reveal their antibiogram patterns and typing. Thirty-two of them were found to be *P. stuartii* and two of them *P. rettgeri*. Tests

for antibiotic sensitivities were performed by conventional disk diffusion method according to the recommendations of NCCLS (National Committee of Clinical Laboratory Standards). A comparison of antibiogram patterns of *Providencia* isolates revealed two identical clusters; six *P. stuartii* and one *P. rettgeri* were seen in the first cluster and seven *P. stuartii* isolates were observed in the second. These findings could indicate that either a cross infection had occurred among different patients or genomic transfer of resistance genes had taken place among different strains in hospital environment.

Key Words: *Providencia* spp, Nosocomial urinary tract infection, Long-term urinary catheterization, Catheter-associated bacteriuria

Introduction

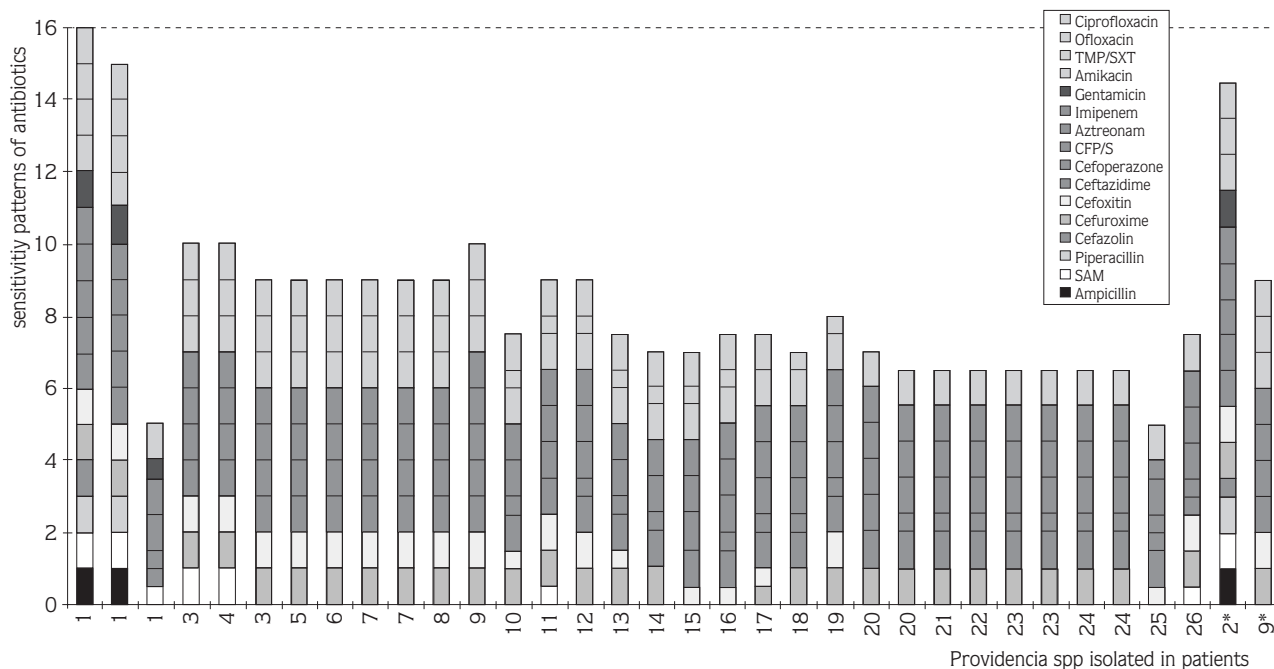
Patient profiles in rehabilitation centres are quite different from other centres. In the USA, inpatients with spinal cord injury and urinary incontinence necessitating of long-term catheterization, nosocomial urinary tract infections (UTIs) are reported as quite common. 1,2 *Providencia* spp, despite a rare uropathogen in community-acquired UTIs and uncatheterized patients, have a high prevalence among long-term catheterized patients. A niche for *Providencia* occurs after long-term catheterization of the urinary tract. The most common reported agent from nosocomial UTIs or colonization is *P. stuartii*. Even though it is mostly asymptomatic, mortality and morbidity levels are increased. 2-6 Bacteria frequently show multiple-antibiotic resistance therefore, it is important to note that occasionally bacteremia secondary to UTI and death may occur. 7,8,9

In this study, antibiotic susceptibility pattern of *Providencia* spp isolates of inpatients in a rehabilitation centre are compared and reported. The possibility of cross infection with clusters of isolates having the same strain are discussed.

Material and Methods

Specimen source and handling: Between October 1993 and April 1994, a total of 428 urine samples was obtained from hospitalized patients in the Ankara Rehabilitation Centre. They were submitted to the Bacteriology Laboratory at the National Central Hygiene Institute for microbiological diagnosis. The specimens were mostly taken from spinally-injured inpatients with long-term catheterization (>30 days). After clamping the catheter, each sample was collected aseptically from the proximal portion of the urethral catheter with a sterile injector, within half an hour they were sent to the laboratory. Clinical evidence of symptomatic nosocomial urinary tract infection was based on criteria from CDC definitions including one of the following symptoms; fever (>38°C), urgency, dysuria or suprapubic tenderness and a urine culture of $\geq 10^5$ colonies/ml urine with no more than two species of organisms.¹⁰

Culture of urine samples: Urine samples were inoculated on 5% sheep blood agar (Blood Agar Base No: 2, Oxoid) and EMB agar(Oxoid) with a technique



*: *Providencia rettgeri* isolated patients Values: Resistance=0, Intermediate= 0.5, Sensitivity= 1

Figure 1. A profile of antibiotic sensitivities of 34 *Providencia* strains isolated from 26 patients

capable of detecting ≥ 1000 colony forming units (cfu) per milliliter by a standard loop of 10 μ l. All plates were examined after overnight incubation at 37°C. Urine samples which grew more than 10^5 cfu/ml were accepted as significant bacteriuria.^{10,11} Three hundreds and thirty-one specimens were found positive for bacteriuria. All isolates were studied for further identification by conventional methods (passages to the Kligler Iron Agar, IMViC reactions, urease activity, lactos, mannitol, adonitol, trehalose fermentation, phenylalanine and arginin deamination, ornithine and lysine decarboxilation tests). Forty of these strains (40/331) were determined as *Providencia spp* that they also confirmed by API 20E strips (bioMerieux). The distribution of bacteriuria agents is summarized in Table 1.

Antimicrobial susceptibility testing: In this study, the main aim is to reveal whether there is a relation between *Providencia* isolates and antibiotic susceptibility patterns. Therefore, all antibiograms of *Providencia* strains were tested for typing that was based on study of Giacca et al.¹² After primary isolation, *Providencia* isolates were frozen in tryptic soy broth (Oxoid) with 20% glycerol (Merck) at -20°C until performing antimicrobial susceptibility tests (approx. 8-12 weeks). During the period of preservation, six strains

Table 1. Distribution of microorganisms and ratios of positive cultures in urinary samples obtained from inpatients with long-term catheterization.

Microorganism	Positive urine cultures ($\geq 10^5$ cfu/ml)	
	n	%
<i>Eshcherichia coli</i>	80	24.2
<i>Proteus spp.</i>	64	19.3
<i>Pseudomonas aeruginosa</i>	53	16.0
<i>Klebsiella spp.</i>	53	16.0
<i>Providencia spp.</i>	40	12.1
Gram negative enteric bacilli (others)	22	6.7
Gram positive coccus	10	3.0
Micellaneous bacteria	2	0.6
<i>Candida albicans</i>	7	2.1
TOTAL	331	100.0

were lost. The remaining thirty-four *Providencia spp* were studied. Antibiograms were performed by the conventional disk diffusion, NCCLS standardized method¹³, on Mueller-Hinton Agar medium (Oxoid). At first, each isolate was inoculated in Mueller-Hinton

Table 2. The antibiogram profiles of *Providencia* spp arranged to the samples' submitting date.^a

Date	Protocol No:	Patients	Ampicillin	SAM	Piperacillin	Cefazolin	Cefuroxime	Cefoxitin	Ceftazidime	Cefoperazone	CFP/S	Aztreonam	Ampicillin/impipenem	Gentamicin	Amikacin	TMP/SXT	Ofloxacin	Ciprofloxacin
2.1.1994	9	1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2.1.1994	30	18	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	I
3.1.1994	38	7	R	R	R	R	S	S	S	R	S	S	S	R	S	R	S	S
3.1.1994	39	3	R	S	R	R	S	S	S	R	S	S	S	R	S	R	S	S
5.1.1994	82	1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
13.1.1994	90	24	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	R
17.1.1994	96	9	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	R
17.1.1994	97	6	R	R	R	R	S	S	S	R	S	S	S	R	S	R	S	S
19.1.1994	104	1	R	R	R	R	R	I	I	R	I	S	S	I	S	R	R	R
25.1.1994	118	23	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	R
1.2.1994	140	24	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	R
1.2.1994	143	2 ^b	S	S	S	I	S	S	S	S	S	S	S	S	S	R	S	S
1.2.1994	144	19	R	R	R	R	S	S	S	I	S	S	S	R	S	R	R	I
3.2.1994	171	23	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	R
3.2.1994	173	7	R	R	R	R	S	S	S	R	S	S	S	R	S	R	S	S
3.2.1994	174	4	R	S	R	R	S	S	S	R	S	S	S	R	S	R	S	S
3.2.1994	176	5	R	R	R	R	S	S	S	R	S	S	S	R	S	R	S	S
15.2.1994	200	20	R	R	R	R	S	R	S	S	S	S	S	R	S	R	R	R
15.2.1994	203	9 ^b	R	R	R	R	S	S	S	R	S	S	S	R	S	R	S	S
15.2.1994	204	14	R	R	R	R	S	R	S	R	I	S	S	R	S	R	I	S
17.2.1994	230	3	R	R	R	R	S	S	S	R	S	S	S	R	S	R	S	S
18.2.1994	231	11	R	I	R	R	S	S	S	R	S	S	S	R	S	R	I	S
18.2.1994	232	12	R	R	R	R	S	S	S	I	S	S	S	R	S	R	I	S
18.2.1994	250	26	R	I	S	R	R	S	I	I	S	S	S	R	S	R	R	R
24.2.1994	267	15	R	R	R	R	R	I	S	R	S	S	S	R	S	R	I	S
24.2.1994	268	22	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	R
24.2.1994	269	21	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	R
25.2.1994	287	8	R	R	R	R	S	S	S	R	S	S	S	R	S	R	S	S
25.2.1994	288	17	R	R	R	R	I	I	S	I	S	S	S	R	S	R	R	S
28.2.1994	291	13	R	R	R	R	S	I	S	R	I	S	S	R	S	R	I	S
8.3.1994	329	25	R	R	R	R	R	I	S	I	I	S	I	R	S	R	R	R
24.3.1994	363	16	R	R	R	R	R	I	S	I	S	S	S	R	S	R	I	S
30.3.1994	384	20	R	R	R	R	S	R	S	I	S	S	S	R	S	R	R	R
30.3.1994	401	10	R	R	R	R	S	I	S	R	I	S	S	R	S	R	I	S
		S%	8.8	14.7	11.8	2.9	82.5	50	94.1	14.7	85.3	100	97.1	8.8	100	5.9	38.2	61.8

■ : ■ : Similar antibiogram pattern groups

S: Susceptible I: Intermediate, R: Resistant

a: Six of the patients had two samples and one of the patients had three samples which isolated *Providencia* spp at different time

b: *Providencia rettgeri* isolated patients

broth and incubated at 37°C until a slightly visible turbidity appears (2 to 5 hours). The turbidity was then adjusted with saline to obtain it visually comparable to McFarland 0.5 standard.¹³ Sixteen antibiotics, ampicillin, sulbactam-ampicillin (SAM), piperacillin, ceftazidime, cefuroxime, ceftazidime, cefoperazone, cefoperazone-sulbactam (CFP/S), aztreonam, imipenem, gentamicin, amikacin, trimethoprim-sulfamethoxazole (TMP-SXT), ofloxacin and ciprofloxacin, were tested. After overnight incubation at 37°C disk zone sizes were measured with a ruler and entered into the database. *Escherichia coli* ATCC 25922 was used for quality control. When the antibiogram was studied the patients were renumbered.

Data analysis: For every strain, the measurements of zone sizes were interpreted and given the following values; resistance 0, susceptibility 1, and intermediate 0.5. All findings were transferred to a data sheet and enumerated (Figure 1).

Results

During the course of the study, three hundreds and thirty-one urine samples (77.3%) were found to be positive for bacteriuria. The species of the isolates and the incidences were shown in Table 1. Forty isolates (12.1%) were determined as *Providencia* spp. They had the fifth highest frequency among urinary infection agents in long-term catheterized patients (Table 1). *Escherichia coli*, the highest agent, was isolated from eighty of the cases (24.2%).

In the course of the study, six isolates were lost during the preservation. The remaining 34 *Providencia* isolates were further investigated, so as to find their relation with the antibiogram patterns. Thirty-two of them were *P. stuartii* and two of them *P. rettgeri*. These 34 isolates were obtained from 26 patients; more than one sampling was taken from seven patients at different times and significantly each sample was found to have *Providencia* spp. There was a minimum of three days and a maximum of 45 days between sample taking. The antibiogram profiles of *Providencia* spp that were arranged according to the samples submitting date are shown in Table 2. All *Providencia* spp isolated patients had long-term catheterization (>30 days). Eight cases had urolithiasis. The mean age was 28±14.5(6-61 years) in 23 males and three females. The patients had no bladder infection when admitted to the hospital.

The antibiotic susceptibility profiles of *Providencia*

strains are shown in Table 2. When antibiotic patterns of all strains are compared, two groups are seen to be similar. The first group comprises six *P. stuartii* and one *P. rettgeri*, whereas the second group has seven *P. stuartii* and no *P. rettgeri* strains (Figure 1, Table 2).

Discussion

E.coli was the highest causative agent of nosocomial UTIs (24.2%) in our results. *Proteus* spp (19.3%), *P.aeruginosa* (16.0%) and *Klebsiella* spp (16.0%) followed respectively (Table 1). Our results are similar with the other comparable studies.^{2,6} However, in our study *Providencia* spp was found to be higher than in other studies in Turkey. It was the fifth causative agent (12.1%), while in other similar studies it had been shown as the lowest causative agent (0-2.4%).¹³⁻¹⁶

Providencia spp, particularly *P. stuartii*, is a common agent of nosocomial UTI agents in patients with chronic urethral catheterization. Patients who have spinal cord injury with urologic disorders are the highest risk group for *Providencia* strains. These bacteria can cause fatal bacteremia and they have multiple-resistance to antibiotics.²⁻⁹

In our laboratory, we compared the isolation rate of *Providencia* between 428 inpatients' and 1505 outpatients' urine samples at the same time over an eight month period. From the outpatients' samples three hundreds and thirteen had significant bacteriuria and only one of them was determined as *Providencia* spp (0.3%).¹⁷ This difference was found to be statistically significant (p<0.001).

If an organism passes from one inpatient to another in an hospital environment it is termed as cross colonization/infection.¹⁸ In our study, the existence of two large groups with similar antibiograms suggests an endemic-epidemic cross infection or genomic transfer between *Providencia* strains in an hospital environment. One of these clusters, which includes six *P. stuartii* strains and one *P. rettgeri*, indicates a strong possibility that this transfer may be carried out interspecies.

In these clusters, two sampling were taken from three patients (*Patient 7, Patient 23, Patient 24*) and showed isolated *P.stuartii* at different times. These strains had similar antibiograms, even though, here was a minimum of nine and a maximum of 30 days (Figure 1, Table 2). The period of bacteriuric episodes

caused by *P. stuartii* is remarkably longer than other bacteriuric episodes, except for *E.coli*. This situation depends on long bacterial persistence in the urinary tract, moreover it can be isolated after weeks or months from the first isolation.⁷ High numbers of *P.stuartii* can be isolated due to its long persistence in the urinary tract not to long term urinary catheterization. Thus, the isolation of *Providencia spp* from patients with short term or no catheterization is rare.^{3,7}

In the course of the study, we detected two small clusters only seen in four patients (*Patient 3 and Patient 4, Patient 10 and Patient 13*). Interestingly, sampling was taken approximately 30 days apart (Figure 1, Table 2).

The relation between different catheter types and adherence was studied by Roberts et al.¹⁹ They found that the greatest adherence is to the red rubber catheters, but no bacterial adherece to the hydrophilic catheter surfaces was found. In our study, the use of red rubber catheter in all patients and the period of catheterization, more than 30 days, may also correlate with the detection of recurrent *Providencia spp* bacteriuria in seven cases.

However, several reports indicate that multiple-antibiotic resistance is encoded by the plasmids in *Providencia spp*, especially *P.stuartii* serves as a reservoir for the plasmids responsible for the transfer of multiple-antibiotic resistance. ^{3,7} Although *P.stuartii* is not a highly pathogenic microorganism, it has a great por-

tance in the transfer of potential virulence factors from other bacteria and multiple antibiotic resistance patterns.^{7,8} This could indicate that *Patient 1* had three *P.stuartii* strains with different antibiograms. There were a minimum of three and a maximum of 15 days between urine sample collection from *Patient 1*. *Patient 3* also had two strains of *P. stuartii* with different antibiogram patterns. On the other hand, *Patient 9* had two different species of *Providencia* (*P.stuartii* and *P. rettgeri*) which they obtained from two urine samples that sampling 30 days part (Table 2).

As a member of *Enterobacteriaceae*, *Providencia* species, due to poor past descriptions and taxonomic problems, just began to be reported in the 1970's. A tremendous amount of investigation showed the role of *P. stuartii* in nosocomial UTIs, particularly with long-term catheterization. We, therefore, investigated any relationship between *Providencia* species and cross-infection by antibiogram patterns. We also suggest support the usage of antibiogram patterns method as many other studies the purpose of typing in hospital routine laboraroties. ^{12,18} This method can be easily applied to obtain epidemiological data over certain periods. Thus, in order to decrease infection morbidity and increase the quality of life in catheterized patients; the prevalence, distribution and antibiotic susceptibility patterns of this microorganism must be monitorized periodically in rehabilitation centers, and in other relevant hospital units.

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