Investigation of Antibiotic Resistance Rates of *Providencia stuartii* isolated from Various Clinical Samples

Bahadır Feyzioğlu, Mehmet Özdemir, Metin Doğan, Mahmut Baykan, Bülent Baysal

Department of Medical Microbiology, Necmettin Erbakan University Meram Faculty of Medicine, Konya, Turkey
Corresponding author: *Assistant Professor* Bahadır Feyzioğlu
Address: Department of Medical Microbiology, Necmettin Erbakan University, Meram Faculty of Medicine, Konya, Turkey
Phone: +90 332 223 7347, Fax: +90 332 223 6181
E-mail: drbhdr@gmail.com

ABSTRACT

Objective: Distribution of 85 *Providencia stuartii* strains isolated between June 2011 and June 2013 to clinical samples, to clinics that the samples were sent from, and the antibiotic susceptibility of the strains were aimed. **Material and Method:** Identifications and antibiotic susceptibility of the *Providencia stuartii* isolates were performed by conventional methods and VITEK 2 (bioMerieux, France) automated system, according to the standards of The European Committee on Antimicrobial Susceptibility Testing. **Results:** *Providencia stuartii* strains were mainly isolated from intensive care units. Sample distribution of the bacteria was as follows: 51,76 % from sputum, 11,76 % from blood, 10,59 % from urine, 9,41 % from catheter and the remaining 16.47 % from other samples. The susceptibility of *Providencia stuartii* strains were determineted as 76 % for meropenem, 16.6 % for piperacillin-tazobactam, 11 % amikacin, 8.5 % for imipenem, 4 % for ciprofloxacin, and 2.6 % for piperacillin. **Conclusion:** As a result, meropenem were found to be the most effective antibiotics for *Providencia stuartiiin*. High rates of resistance to beta lactams, quinolones, aminoglycoside and other antibiotics were also arresting.

Key words: Antibiotic Resistance, ESBL meropenem, Providencia stuartii

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INTRODUCTION

The genus Providencia are responsible for a wide range of human infections, but they are rare and usually nosocomial. The first species of the genus now known as Providencia was isolated by Rettger in 1904. There are 5 species of the genus Providencia. In descending order of prevalence, include *Providencia stuartii, Providencia rettgeri, Providencia alcalifaciens, Providencia rustigianii*, and *Providencia heimbachae* (1).

Providencia stuartii is a gram stain negative, facultative anaerobe and motile bacteria. It is found in multiple animal reservoirs such as flies, birds, cats, dogs, cattle, sheep and is also found commonly in soil, water, and sewage. It is an opportunistic pathogen seen in patients with severe burns or long-term indwelling urinary catheters. *Providencia stuartii* outbreaks are usually of urinary origin. This species have been isolated from urine, stool, and blood, as well as from sputum, skin, and wound cultures.

Providencia stuartii is the least susceptible to antimicrobials in Providencia sp. and is naturally resistant to tetracyclines, some penicillins, older cephalosporins, sulphamethoxazole and fosfomycin (2). However, the acquired resistance is an increasing problem. *Providencia stuartii* can be multiresistant to antibiotics especially to beta-lactams. Especially ESBL-positive strains are problem in hospitalized patients (3,4). The occurence of resistance to quinolones and aminoglycosides has caused difficulties in the treatment, in recent years

(5,6,7). Recently, reported studies have expressed that the increase in infections of Providencia *stuartii* had been associated with the increased use of some antibiotics such as colistin (8). The number of infections caused by this bacterium has increased day by day, but there are a few reports about the antibiotics resistance of *Providencia stuarti*. Probably, the infections of *Providencia stuarti* will be more frequent in the future. For accurate and reliable treatment, we need to know that the effectiveness of antibiotics against to this species. In this study, we examined that the effectiveness of antibiotics against to considerable number of strains.

MATERIALS AND METHODS

This study was conducted from June 2011 to June 2013 in a university hospital in Turkey. Variety clinical samples from suspected cases of infection were processed by microscopy and culture. A total of 85 isolates of *Providencia stuarti* were studied for the antimicrobial susceptibility patterns.

Method of collection

The specimens were collected before the administration of antibiotics. The sputum / BAL, blood, urine, catheter, sterile body fluids, tracheal aspirates, wound and throat specimens were taken as defined in the guidelines (9). The specimens were immediately transported to the laboratory and processed.

Isolation and Identification of *Providencia stuarti*

For isolation, 5 % Sheep blood agar (bioMérieux, Marcy L'Etoile, France) and EMB agar (bioMérieux, Marcy L'Etoile, France) were used. The isolation and identification of the

bacteria was carried out by both automated Vitek 2 system (BioMérieux) and conventional methods such as, gram staining, pigment production, catalase, oxidase, citrate, urease, three sugars fermenting, indole and motility tests. Then, isolates were confirmed as *Providencia stuarti* and antibiotic susceptibility testing was carried out.

Susceptibility testing

Susceptibility testing was performed on Mueller–Hinton agar (bioMérieux, Marcy L'Etoile, France) using McFarland 0.5 with overnight cultures and incubated at 35°C for 16–18 h. For susceptibility testing the disc diffusion method according to Kirby–Bauer was used and Minimal inhibitory concentrations (MIC) were determined by the agar dilution technique with the following antibiotics: ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin, piperacillin-tazobactam, ceftazidime, cefuroxime, cefoxitin, imipenem, meropenem, ciprofloxacin, amikacin, gentamicin, tigecycline. *P. aeruginosa* ATCC 27853 used as a positive control. The results were interpreted according to the 2011 guidelines of The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (10).

RESULTS

In the study, A total of 85 *P. stuartii* were isolated. They were isolated most frequently from reanimation unit followed by intensive care unit of chest diseases (Table-1). The maximum number of *P. stuartii* was isolated from BAL/Sputum (51,76%), followed by blood (11.76%) (Table-2). A large part of strains were sensitive to meropenem, but not imipenem. Also, the higher resistance to piperacillin-tazobactam, amikacin, and ciprofloxacin was seen. The antibiotic susceptibility of the isolated strains has been shown in Table 3.

Table 1. The distribution of the isolates according to the clinics

Servis	n (%)
Reanimation	23 (27.06)
Chest diseases (intensive care)	19 (22.35)
Chest diseases (service)	8 (9.40)
Surgical clinics *	7 (8.24)
Pediatric intensive Care	7 (8.24)
Internal Service	7 (8.24)
Department of Urology	6 (7.06)
Department of Neurology	3 (3.53)
Radiation Oncology	3 (3.53)
Pediatric Diseases	2 (2.35)

* General Surgery, Pediatric Surgery, Plastic Surgery, Emergency Surgery, Cardiovascular Surgery, Neurosurgery

Table 2. The distribution of clinical specimens

Örnek	n (%)
Sputum / BAL	44 (51.76)
Blood	10 (11.76)
Urine	9 (10.59)
Catheter	8 (9.41)
Sterile body fluids *	5 (5.88)
Tracheal aspirate	4 (4.71)
Wound swab	3 (3.53)
Throat swab	2 (2.35)

BAL, bronchoalveolar lavage

* Pleural fluid, peritoneal fluid, cerebrospinal fluid

Table 3. The MIC values in *P.sturatii* isolates

ANTIBIOTICS	sensitivity rate%	MIC values (mg/L)												EUCAST criteria		
		0.25	0.5	1	2	4	8	16	32	64	128	256	512	S≤	R>	ECOFF≤
Meropenem	**76**	0	**41**	**14**	**2**	**2**	**10**	**6**	0	0	0	0	0	**2**	**8**	**0.25**
Piperacillin-tazobactam	**16.6**	0	0	0	**1**	**4**	**8**	**3**	**1**	**1**	**12**	0	0	**8**	**16**	**8**
Amikacin	**11**	0	0	0	**8**	0	**1**	**8**	0	**65**	0	0	0	**8**	**16**	*
Imipenem	**8.5**	0	0	**1**	**6**	**22**	**2**	**50**	0	0	0	**1**	0	**2**	**8**	**4**
Ciprofloxacin	**4**	0	**3**	0	**2**	**69**	0	0	**1**	0	0	0	0	**0.5**	**1**	*
Piperacillin	**2.6**	0	**2**	0	0	0	**3**	**2**	**46**	0	**23**	0	0	**8**	**16**	*
Amoxicillin-clavulanic acid	**0**	0	0	0	0	0	0	0	**19**	0	0	0	0	**8**	**8**	*
Ampicillin	**0**	0	0	0	0	0	0	0	**19**	0	0	0	0	**8**	**8**	*
Ampicillin-sulbactam	**0**	0	0	0	0	0	0	0	**23**	0	**38**	0	0	**8**	**8**	*
Ceftazidime	**0**	0	0	0	0	**2**	0	**1**		**79**	0	0	0	**1**	**4**	**0.5**
Gentamicin	**0**	0	0	0	0	**1**	0	**64**	0	0	0	**2**	**1**	**2**	**2**	*
Cefoxitin	*	0	0	0	0	0	0	**1**	**1**	**63**	**5**	0	0	*	*	*
Cefuroxime	*	*0*	*0*	*0*	*0*	*0*	***1***	*0*	***1***	***34***	*0*	*0*	*0*	*	*	*
Tigecycline	*	0	0	0	0	0	**25**	**39**	**12**	**2**		0	0	*	*	**8**

S, susceptible, R; resistant, ECOFF; Epidemiological cut-off value,

* undetermined value

DISCUSSION

Providencia stuartii is etiological agent of nosocomial infections. It increases especially in the patients who have medical care. It often leads to urinary tract infections, and causes less frequently respiratory and skin infections. There are predisposing factors such as catheterisation and intubation causing infections with this bacterium. These species have been isolated from urine, stool, and blood, as well as from sputum, skin, and wound cultures. In this study, the more than half of the isolates were isolated from specimens from intensive

care units. We considered that the long-term health care and use of antibiotics caused clustering of infections in these units. Already, the most of the reports have been associated with intensive care units in the literature (4,6,11). It is interesting that, although the bacteria frequently isolated from urine samples in previous reports (7,12), the most number of *P.stuartii isolated* from respiratory samples in our study. The respiratory tract catheterization may be an important predisposing factor for colonization and infection of this bacteria.

The higher MIC values and resistance rates in our isolates is noteworthy. This isolates were resistance to Amoxicillin-clavulanic acid, ampicillin, ampicillin-sulbactam, ceftazidime, and gentamicin, but also we know that, the bacteria is naturally resistant to these antimicrobials. However, the MIC values for piperacillin-tazobactam, amikacin, imipenem, ciprofloxacin, piperacillin, cefoxitin, cefuroxime were higher and the low effectiveness of these antimicrobials was indication of a serious problem. Also, meropenem was the only considerable effective (76%) antimicrobial in our study.

The natural resistance to tetracyclines, fosfomycin, aminoglycosides (especially gentamicin and tobramycin), many of the older generation penicillins and cephalosporins was defined in *P.stuartii* species, previously (2). There are a few reports about multidrug resistant Providencia species, since the 1970s. However, it was observed an increase in hospital infections caused by multi drug resistance isolates with ESBL producted, particularly in recent years (13,14,15). These notifications are concentrated in the Mediterranean and the Balkan countries associated with our country (11,16,17,18).

Meropenem was the most effective antimicrobial to our isolates in our study. However, imipenem MIC values have increased dramatically. The strains which resistant to imipenem but not resistant to meropenem emerge as a result of OprD porin loss in *P.aeruginosa*. It may be considered that, the presence of similar mechanisms to explain this

situation. The porin protein loss is not enough for resistance to meropenem via efflux pumps, also, the mutations on the active outward pumping system should be present (19). On the other hand, carbapenemase such as class A penicillinase, class B metallo-beta-lactamase and class D oxacillinase likely caused resistant to imipenem and meropenem in our isolates. It was reported that, resistance to all beta-lactams except imipenem occured by the mutation of mexAB-oprM multidrug efflux system in *P. Aeruginosa*. The expression of MexEF-OprN efflux pump and oprD porin is co-regulated. It's is a known fact that a substitution simultaneously affected these regions may cause resistance to imipenem and quinolone but not meropenem species (20). This phenomenon can be considered as a mechanism for our some of isolates. The occuring of resistance via efflux pumping system was commonly described mechanisms for the quinolone resistance in recent years. According to our results, MIC values for ciprofloxacin increased in the majority of isolates. The natural resistance to quinolones is rare in Providencia species. Nonetheless, there are higher MIC values for *P.stuartii* when it was compared to other species in the genus. The quinolone resistance occurs as a result of successive mutations in the target enzyme in bacteria. As a result of mutation of the target molecule, the second molecule becomes relatively further susceptible to the drug. This second molecule mutated after repeated uses of the drug and this event continues alternately from a molecule to another. Thus, the exponentially increased MIC values ocur. The plasmid-mediated quinolone resistance has been frequently emphasized subject in recent times and it has been reported with increasing frequency for Enterobacteriaceae (21,22). Some of the genes transferred by plasmids (qnr, qepA) lead to singly an increase for quinolone MIC values. Whereas some of the expressed genes related to aminoglycoside resistance (aac (6 ')-Ib-cr) or multi-drug resistance (oqxAB) (23,24,25). Most of our isolates have multiple antibiotic resistance profile and if we consider the information related to carbapenem resistance in above discussion; these multi-drug resistance isolates may

have occurred by inducible pumping system mechanisms. All of the isolates showed resistance to gentamicin, and amikacin was effective to 11% of the strains. The natural resistance has been shown for gentamicin in *P.stuartii* wild strains, but hasn't been defined for amikacin. On the other hand, there is a acquired resistance to amikacin (2). It is known that the aminoglycoside resistance in gram-negative bacteria usually occurs through aminoglycosides altering enzymes. In previous reports, it has been shown AAC (2 ')(2'-N-acetyltransferase) resistance profile in Providencia species, and this stuation appears as the reason of resistant to gentamicin but not to amikacin. On the other hand, we considered that the higher MIC values of amikacin in our isolates may be related to the AAC (6') (6'-N-acetyltransferase type) mechanism previously described in enteric bacteria (3,26).

The notifications of nosocomial infection related to *Providenci stuartii* increased remarkably in our nearby geography in recent years. There are a few reports on epidemiological data and antibiotic susceptibility rates of this bacteria. The high MIC values and multi-drug resistance were determined in our isolates. The antibiotics have the effect of inducing for the emergence of drug resistance during the empirical treatment, so accurate identification and antibiogram for this bacteria is extremely important. We have determined phenotypic resistance profiles in our study. We believe that our results will offer a significant contribution to antibiotic treatment strategies for *Providencia stuartii* infections increasing day by day.

REFERENCES

1. Manos J, Belas R. The Genera Proteus, Providencia, and Morganella. Prokaryotes. 2006;6:245–69.

2. Stock I, Wiedemann B. Natural antibiotic susceptibility of Providencia stuartii, P. rettgeri, P. alcalifaciens and P. rustigianii strains. J Med Microbiol. 1998;47:629-42.
3. Whiteley GR, Penner JL, Stewart IO, Stokan PC, Hinton NA. Nosocomial urinary tract infections caused by two O-serotypes of Providencia stuartii in one hospital. J Clin Microbiol. 1977;6:551-4.
4. Kocka FE, Srinivasan S, Mowjood M, Kantor HS. Nosocomial multiply resistant Providencia stuartii: a long-term outbreak with multiple biotypes and serotypes at one hospital. J Clin Microbiol. 1980;11:167-9.
5. Lambert T, Gerbaud G, Courvalin P. Characterization of transposon Tn1528, which confers amikacin resistance by synthesis of aminoglycoside 3'-O-phosphotransferase type VI. Antimicrob Agents Chemother. 1994 April; 38(4): 702–706.
6. Nazik H, Bektöre B, Öngen B, Özyurt M, Baylan O, Haznedaroglu T: Co-expression of plasmid-mediated quinolone resistance-qnrA1 and blaVEB-1 gene in a Providencia stuartii strain. New Mıcrobıologıca, 34, 225-228, 2011.
7. Tumbarello M, Citton R, Spanu T, Sanguinetti M, Romano L, Fadda G, Cauda R. ESBL-producing multidrug-resistant Providencia stuartii infections in a university hospital. J Antimicrob Chemother. 2004 Feb;53(2):277-82.
8. Hayakawa K, Marchaim D, Divine GW, Pogue JM, Kumar S, Lephart P, Risko K, Sobel JD, Kaye KS. Growing prevalence of Providencia stuartii associated with the increased usage of colistin at a tertiary health care center. Int J Infect Dis.2012;16:646-8.
9. Clinical Microbiology Standards Committee. Sample Collection Procedure Manual. Diagnostic Services Of Manitoba Inc – Microbiology. 2012 May;1-61

10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M 100-S21. Wayne, PA, USA: CLSI; 2011

11. Mnif B, Ktari S, Chaari A, Medhioub F, Rhimi F, Bouaziz M, Hammami A. Nosocomial dissemination of Providencia stuartii isolates carrying bla OXA-48,bla PER-1, bla CMY-4 and qnrA6 in a Tunisian hospital. J Antimicrob Chemother. 2013 Feb;68(2):329-32.

12. Rahav G, Pinco E, Silbaq F, Bercovier H. Molecular epidemiology of catheter-associated bacteriuria in nursing home patients. J Clin Microbiol. Apr 1994;32(4):1031-4

13. Overturf GD, Wilkins J, Ressler R. Emergence of resistance of Providencia stuartii to multiple antibiotics: speciation and biochemical characterization of Providencia. J Infect Dis. 1974 Mar;129(3):353-7

14. Kaslow RA, Lindsey JO, Bisno AL, Price A. Nosocomial infection with highly resistant, Proteus rettgeri. Report of an epidemic. Am J Epidemiol. 1976 Sep;104(3):278-86,

15. Warren, J. W. 1986.Providencia stuartii: a common cause of antibiotic resistant bacteriuria in patients with long-term indwelling catheters. Rev.Infect. Dis. 8:61–67

16. Lahlaoui H, Poirel L, Moussa MB, Ferjani M, Omrane B, Nordmann P. Nosocomial dissemination of extended-spectrum β-lactamase VEB-1a-producing Providencia stuartii isolates in a Tunisian hospital. Eur J Clin Microbiol Infect Dis. 2011 Oct;30(10):1267-70.

17. Saida NB, Thabet L, Messadi A, Bouselmi K, Turki A, Boukadida J. Clonality of Providencia stuartii isolates involved in outbreak that occurred in a burn unit. Burns. 2008;34:829-34

18. Arnoldo L, Migliavacca R, Regattin L, Raglio A, Pagani L, Nucleo E, Spalla M, Vailati F, Agodi A, Mosca A, Zotti C, Tardivo S, Bianco I, Rulli A, Gualdi P, Panetta P, Pasini C, Pedroni M, Brusaferro S. Prevalence of urinary colonization by extended spectrum-beta-lactamase Enterobacteriaceae among catheterised inpatients in Italian long term care facilities. BMC Infect Dis. 2013;13:124

19. Nikaido H. Antibiotic resistance caused by gram-negative multidrug efflux pumps. Clin Infect Dis. 1998 Aug;27 Suppl 1:S32-41.

20. Ochs MM, Lu CD, Hancock RE, Abdelal AT. Amino acid-mediated induction of the basic amino acid-specific outer membrane porin OprD from Pseudomonas aeruginosa. J Bacteriol. 1999 Sep;181(17):5426-32.

21. Investigation of Plasmid-Mediated Quinolone Resistance Determinants in Enterobacteriaceae: A Multicenter Study. Mikrobiyol Bul 2012; 46(3): 366-374

22. Strahilevitz J, Jacoby GA, Hooper D, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. Clin Microb Rev 2009; 22(4): 664-89.

23. Martinez LM, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet 1998; 351(9105): 797-9.

24. Yamane K, Wochino J, Suzuki S, et al. New plasmid-medited quinolone efflux pump, QepA, found in an Escherichia coli clinical isolate. Antimimicrob Agents Chemother 2007; 51(9): 3354-60.

25. Herrera-Leon S, Gonzalez-Sanz R, Herrera-Leon L, Echeita MA. Characterization of multidrug resistant Enterobacteriaceae carrying plasmid-mediated quinolone resistance mechanisms in Spain. J Antimicrob Chemother 2011; 66(2): 287-90.

26. Franklin K, Clarke AJ. Overexpression and characterization of the chromosomal aminoglycoside 2'-N-acetyltransferase of Providencia stuartii. Antimicrob Agents Chemother. 2001;45:2238-44