European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 25-01-2020; Accepted: 10-02-2020; Published: 26-02-2020
www.biosciencejournals.com
Volume 8; Issue 1; 2020; Page No. 43-47

Antimicrobial Sensitivity Patterns of *Proteus mirabilis* İsolates from Urine Samples

D Keskin[1*], S Kirac[2], M Yarar[3]
[1] Kosk Vocational High School, Adnan Menderes University, Kosk-Aydin, Turkey
[2] Denizli Health Services Vocational High School, Pamukkale University Denizli, Turkey
[3] Denizli State Hospital, Denizli, Turkey

Abstract
Objective: To assignation the grade of resistance to the widely used antibiotics in clinical isolates of *Proteus mirabilis* 60 isolates were collected from special hospital in Denizli and recorded at specimens.
Method: Antibiotic resistance was determined by agar disc diffusion method using Mueller-Hinton agar according to Clinical and Laboratory Standards Institute recommendations.
Results: All isolates were defined as Trimetroprim/ Sulfamethaxol and Nitrafurantoin resistant. The resistance rates for Meropenem, Cefixime, and Ceftazidime were 95%, 90% and 87% respectively. 53 isolates (88%) isolates showed Multiple Antibiotic Resistance three to thirteen antibiotics.
Conclusion: Resistance of Proteus to antibiotics was due to selection for drug resistance has been associated with an increased and inappropriate use of antibiotics. There is an irregular use of antimicrobial agents in Turkey.

Keywords: *Proteus mirabilis*, antibiotic sensitivity, urine samples

Introduction
Proteus is a genus of Gram-negative bacteria belonging to the family of Enterobactericeae. Proteus species are distinguishable from most other genera by their ability to swarm across an agar surface [1]. Proteus is widespread in the environment and makes up part of the normal lora of the human gastrointestinal tract. Proteus ranks third as the cause of hospital-acquired infections [2]. Three species: P. vulgaris, P. mirabilis, and P. penneri are opportunistic human pathogens [3]. Proteus species are the major cause of diseases acquired outside the hospital, where many of these diseases eventually require hospitalization [4]. P. mirabilis causes 90% of Proteus infections. Proteus species, particularly P. mirabilis, is believed to be the most common cause of infection-related kidney stone, one of the most serious complications of unresolved or recurrent bacteruria [5]. P. mirabilis has been implicated in meningitis, empyema, osteomyelitis and gastroenteritis. Also, it frequently causes nosocomial infections of the urinary tract (46%), surgical wounds (24%) and lower respiratory tract (30%). Less frequently, proteus species cause bacteraemia (17%), most often in elderly patients [6].
The aim of this study was to determine the characteristics and patterns of antibiotic resistance among isolates of P.mirabilis recovered from urine samples in Denizli.

Subject and methods
Culture and Identification
Total of 60 samples were collected from patients attending Denizli State Hospital Laboratories. Samples were of urine. Isolates were identified depending on morphological and biochemical tests as compared with identification scheme described by [7], and according to API 20E confirmatory test. The specimens were directly streaked onto macconkey and blood agar sand were incubated at 37ºC for 24 hours. In total, 60 P.mirabilis were isolated from various urine samples and detected by the VITEK 2 Compact system (BioMerieux, France) at the microbiology laboratory of our hospital

Microscopic and Morphological Identification
After the isolation of bacteria on MacConkey and blood agar, their shape, size, texture and colony arrangement were observed. Single colonies were picked up, stained with gram stain. Finally they were examined under microscope to identify their shape and length.

Antibiogram Pattern of P.mirabilis
Antibiotic resistance was determined by an agar disc diffusion test [8], using Mueller-Hinton agar (Difco) according to Clinical and Laboratory Standards Institute (CLSI) recommendations (9). Fourteen different antibiotics were used. The antibiotics used were selected according to the 2004 NCCLS guidelines. For antibiotic resistance determination, the isolates were grown in Luria- Bertani (LB) broth until the turbidity equal to the 0.5 Mc Farland standart (approximately 108cfu/ml). Cultures were swabbed on to the Mueller–Hinton agar and all isolates were tested against Trimetroprim/ Sulphamethazol(SXT), Nitrafurantoin (NİT), Meropenem (MEM), Cefixime (CFX), Ceftazidime (CAZ), Ampicillin (AM), Imipenem (IPM), Piperacillin/ Tazobactam (TZP), Ceftriaxon(CFT), Amikacin (AN), Ertapenem (ERT), Gentamicin(GEN), Cefuroxime (CFU), Amoxycillin-Clavunat (AMX). The isolates those grown in inoculation were evaluated as resistant, and the others were evaluated as susceptible [9]. The antibiotic discs were

dispensed sufficiently separated from each other so as to avoid overlapping of inhibition zones. The plates were incubated at 37°C, and the diameters of the inhibition zones were measured after 18 h. All susceptibility tests were carried out in duplicate and were repeated twice if discordant results had been obtained.

Multiple Antibiotic Resistance Index

For all isolates, MAR index values were tested according to Krumperman., 1985 and Matyar *et al*.,2008 [10, 11].

Results

All isolates were defined as Trimetroprim/ Sulfamethaxol and Nitrafurantoin resistant. The resistance rates for Meropenem, Cefixime, and Ceftazidime were 95%, 90% and 87% respectively as shown Table1.

Inserted Table1

Out of 60 P. mirabilis strains isolated 53 (88 %) were MDR as shown Table2.

Discussion

Trimetroprim/ Sulfamethaxol sensitivity was seen in 100% isolates in our study. Some researchers have reported Trimetroprim/ Sulfamethaxol sensitivity rate to P.mirabilis in clinical samples [12, 13]. In contrast to our results, Regasa, [13] reported that all of the P.mirabilis isolates showed resistance to Trimetroprim/ Sulfamethaxol. Trimethoprim and sulfamethoxazole are combined together due to their synergism effect on bacteria. It's abroad spectrum bactericidal antimicrobial agent for both gram positive and gram negative bacteria. Trimethoprim is a diaminopyrimidine, whereas sulfamethoxazole is a sulfonamide and the Co-trimoxazole inhibits the synthesis of tetrahydrofolic acid, which is necessary for the synthesis of bacterial nucleic acid along with two components of the drug inhibiting different steps in the folate synthesis pathway [14].

We found that 100% isolates were sensitive to nitrofurantoin in our study. Some researchers have reported nitrofurantoin sensitivity rate to P.mirabilis in clinical samples [12, 15-19]. Several other studies demonstrated that Proteus was among the most common organisms isolated and percentage of resistant to nitrofurantoin is very high [20, 21].

Our rate of meropenem sensitivity was 95% [1, 22, 23]. Our results were similiar to Wang *et al.* [24] who also reported sensitive to meropenem was 100%.

Our rate of cefixime sensitivity was 90%. Some researchers have reported cefixime sensitivity rate to P.mirabilis in clinical samples [17, 23]. Our results were similiar to Narayana-Swamy *et al*., [23] who also reported sensitive to cefixime was 87,5% in Proteus spp. Our rate of ceftazidime sensitivity was 87%. Some researchers have reported ceftazidime sensitivity rate to P.mirabilis in clinical samples [12, 18, 19, 25-28]. Our results were similiar to Passadoura *et al*., [18] who also reported sensitive to ceftazidime was 90,9% in Proteus spp.We found that 85% isolates were sensitive to ampicilin in our study. Some researchers have reported ampicilin sensitivity rate to P.mirabilis in clinical samples [12, 13, 15, 18, 23 25, 27]. Our results were similiar to Al-Bassam and Al-Kazaz., [12] who also reported sensitive to ampicilin was 75%.

Our rate of imipenem sensitivity was 78%. Some researchers have reported imipenem sensitivity rate to P.mirabilis in clinical samples [12,17,18,22,23,25,27]. Our rate of imipenem resistance was 22%. Our results were similiar to Filgona *et al*., [27] who also reported imipenem resistance was 18,8%.

We found that 75% isolates were sensitive to piperacillin/tazobactam in our study. Some researchers have reported piperacillin/tazobactam sensitivity rate to P.mirabilis in clinical samples [19, 28, 29]. Our rate of ceftriaxone sensitivity was 70%. Some researchers have reported ceftriaxone sensitivity rate to P. mirabilis in clinical samples [16, 17, 18, 22, 27, 29, 30, 31]. Our results were higher than previous researchers [17, 29, 31]. But also our results were lower than Passadoura *et al*., [18].

We found that 57% isolates were sensitive to amikacin in our study. Some researchers have reported amikacin sensitivity rate to P.mirabilis in clinical samples [12, 17, 18, 19, 22, 25, 26, 30, 32, 33]. Our results were similiar to Bahashwan and El Shafey., [25] who also reported sensitivity amikacin was 61,4% in Proteus specimens.

Our rate of ertapenem sensitivity was 53%. Some researchers have reported ertapenem sensitivity rate to P.mirabilis in clinical samples [27, 34, 35]. A Our rate of ertapenem resistance was 23%. Our results were similiar to Filgona *et al*., [27] who also reported ertapenem resistance was 18,8%.

Our rate of gentamycine sensitivity was 52%. Some researchers have reported gentamycine sensitivity rate to P.mirabilis in clinical samples [12, 17, 19, 22, 25, 27, 29, 30, 32, 33]. Our results were similiar to Al-Bassam and Al-Kazaz., [12] who also reported sensitive to gentamycine was 50%.

Our rate of cefuroxime sensitivity was 40%. Our results were higher than Alhambra *et al* [34]. But also, our results were lower than Passadoura *et al*., [18]. Our results were similiar to Manisha *et al*., [36] who also reported cefuroxime sensitivity was 60%.

We found that 33% isolates were sensitive to amoxcillin-clavunat in our study. Some researchers have reported amoxcillin-clavunat sensitivity rate to P.mirabilis in clinical samples [17, 23, 28, 37-39]. Our results were similiar to Romanus *et al*., [39] who also reported amoxcillin-clavunat sensitivity was 40%.

53 isolates (88%) isolates showed Multiple Antibiotic Resistance three to thirteen antibiotics as shown Table2. MDR Proteus reported by Feglo *et al*. [30] was 88%, Leulmi *et al*. [40] 61%, Pandey *et al*. [41] 48.86% and Tumbarello *et al*. [42] 36 % (in blood stream infections). Exposure to piperacillin-tazobactam and empirical cephalosporin use have recently been identified as independent risk factors for MDR P. mirabilis UTIs [43].

Proteus species usually show high resistance to commonly used antibiotics [44]. In this study all the P. mirabilis isolated were sensitive to Trimetroprim/ Sulfamethaxol and Nitrafurantoin. The antibiotic susceptibility tests demonstrated that Proteus species have a wide range of resistance to several antibiotics. This could be a result of the extra outer cytoplasmic membrane which contains a lipid bilayer, lipoproteins, polysaccharides and lipopolysaccharides, and of course, abuse and misuse of antibiotics could be part of the contributing factors of resistance to antibiotics. It is advisable that treatment of Proteus urinary tract infection be guided by the sensitivity result since the antibiotic susceptibility pattern of each species, depends on the extent to which the use of the

various drugs has either selected resistant mutant or promoted the transfer of resistance factor from other members of the enterobacteriaceae [45].

Table 1: Antibiotic suspectibility pattern of *P.mirabilis* isolated from urine samples

Antibiotics	Sensitive	Intermediate	Resistance
SXT	60 (100 %)	0(0%)	0(%0)
NİT	60 (100 %)	0(0%)	0(%0)
MEM	57(95%)	2(3%)	1 (2%)
CFX	54(90%)	6(10%)	0 (0%)
CAZ	52(87%)	3 (5%)	5(8%)
AMP	51 (85%)	8(13%)	1 (2%)
IPM	47 (78%)	13 (%22)	0 (0%)
TZP	45 (75%)	6(10%)	9 (15%)
CFT	42(70%)	14(23%)	4 (6%)
AN	34(57%)	26(43%)	0 (0%)
ERT	32(53%)	26(43%)	2 (3%)
GEN	31 (52%)	29(48%)	0 (0%)
CFU	24(40%)	36(60%)	0 (0%)
AMX	20 (33%)	35(58%)	5 (8%)

Abrevations; SXT; Trimetroprim/ Sulfamethaxol,, NİT; Nitrafurantoin, MEM; Meropenem, CFX:, Cefixime, CAZ; Ceftazidime, AMP, Ampicillin, IPM; Imipenem, TZP; Tazobactam/ piperacillin, CFT; Ceftriaxon, AN; Amikacin, ERT, Ertapenem, GEN; Gentamicin , CFU, Cefuroxime AMX, Amoxycillin-Clavunat

Table 2: Number of urine samples and Multiple Antibiotic Resistance Index 60 *P.mirabilis* strains

Total Resistant Antibiotics	Total İsolate Number	Percentage of Isolates	Mar İndex Value
2	7	12%	0,14
3	8	13%	0,21
4	6	10%	0,29
5	7	12%	0,36
6	9	15%	0,43
7	10	16%	0,5
8	7	12%	0,57
9	3	5%	0,64
10	1	2%	0,71
12	1	2%	0,86
13	1	2%	0,93
	Total 60	Total 100	

Conclusion

Resistance of Proteus to antibiotics was due to selection for drug resistance has been associated with an increased and inappropriate use of antibiotics. There is an irregular use of antimicrobial agents in Turkey.

References

1. Jacobsen SM, Stickler DJ, Mobley HL and Shirtliff ME. Complicated Catheter-Associated Urinary Tract Infections Due to Escherichia coli and Proteus mirabilis. Clin Microbiol Reviews. 2008; 21(1):26-59.
2. Stamm WE. Urinary Tract Infections. In Clinical Infectious Disease: A practical approach, Root, K. (ed). P: 649-656. Oxford University Press, 1999. Inc, New York.
3. Guentzel MN. Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus. In: Barron's Medical Microbiology (Barron 's *et al.*, eds.) (4th ed.). 1996. Univ of Texas Medical Branch. De.
4. De Champs C, Bonnet R, Sirot D, Chanal C, Sirot J. Clinical relevance of Pr. mirabilis in hospital patients: A two year survey. J Antimicrob. Chemoth. 2000; 45:537-539.
5. Coker C, Bakare OO, Mobley HLT. H-NS Is a repressor of the P. mirabilis urease transcriptional activator gene ureR. J.Bacteriol. 2000; 128 (9):2649-2553.
6. Mansy MSM. Genomic fingerprinting using random amplified polymorphic DNA for discrimination between P. mirabilis strains. Egypt. J. Biotech. 2001; 9:67-79.
7. Holt JG, Krieg NR, Sneath PHA, Staley JT, and. Williams ST. Bergy's manual of determinative bacteriology. 9th ed. Williams and Wilkins, 1994 Baltimore, USA.
8. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 1966; 45:493-496.
9. CLSI. Performance standards for antimicrobial susceptibility testing, 15th informational supplement. Document M 100- S 15. Clinical and Laboratory Standards Institute, Wayne, PA. 2005. Danish.
10. Krumperman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. App. Environ. Microbiol. 1985; 46:165-170.
11. Matyar F, Kaya A, Dinçer S. Antibacterial agents and heavy metal resistance in Gram-negative bacteria isolated from seawater, shrimp and sediment in Iskenderun Bay, Turkey. Sci. Total Environ. 2008; 407:279-285.
12. Al-Bassam WW, Al-Kazaz AK. The Isolation and Characterization of Proteus mirabilis from Different Clinical Samples. J Biotechnol Res Center. 2013; 7(2):24-30.
13. Regasa B. Drug Resistance Patterns of Bacterial Pathogens from Adult Patients with Pneumonia in Arba Minch Hospital, South Ethiopia. Global Journal of Medical research: c Microbiology and Pathology. 2014; 14:5: Version 1.0 Year.
14. Ramlakhan S, Singh V, Stone J, Ramtahal A. Clinical Options for the Treatment of Urinary Tract Infections in Children. Clin Med. Insights Pediatr. 2014: 8:31-37.
15. Ahmed AA, Osman H, Mansour AM, Musa HA, Ahmed AB, Karrar Z, Hassan HS. Antimicrobial agent resistance in bacterial isolates from patients with diarrhea and urinary tract infection in the Sudan. Am J

Trop Med Hyg. 2000; 63(5-6):259-63.
16. Sharmin S, Alamgir F, Fahmida, Saleh AA. Antimicrobial sensitivity pattern of uropathogens in children Bangladesh. J Med Microbiol. 2009; 3(01):18-22.
17. Biswas R, Rabbani R, Ahmed HS, Satter -Sarker MA, Zafrin N, Rahman MM. Antibiotic sensitivity pattern of urinary tract infection at a tertiary care hospital. Bangladesh Crit Care J. 2014; 2 (1):21-24.
18. Passadouro R, Raquel Fonseca, Felícia Figueiredo, Andreia Lopes, Cristina Fernandes. Evaluation of the antimicrobial suspectibility of community-Acquired urinary tract infection. Acta Med Port. 2014; 27(6):737-742.
19. Singla P, Sangwan J, Garg S, Chaudhary U. Prevalence and Antibiogram of Multidrug resistant Uropathogenic Isolates of Proteus mirabilis in a Teaching Tertiary Care Hospital. Int J Curr. Microbiol App Sci. 2015; 4(12):675-682.
20. Karlowsky JA, Lagacé-Wiens PRS, Simner PJ, DeCorby MR, Adam HR, *et al*. Antimicrobial Resistance in Urinary Tract Pathogens in Canada from 2007 to 2009: CANWARD Surveillance Study. Antimicrob Agents Chemother. 2011; 55:3169-3175.
21. Ladhani S, Gransden W. Increasing antibiotic resistance among urinary tract isolates. Arch Dis Child. 2003; 88:444-445.
22. Ciftci İH, Asik G, Caliskan K, Cetinkaya Z, Aktepe OC. Antimicrobial Susceptibilities of Proteus Strains Isolated from Clinical Specimens. Ankem J. 2009; 23(3):106-109.
23. .Narayana-Swamy N, Padmasri Ramalingappa P, Urvashi Bhatara U. Antimicrobial Sensitivity Pattern of Microorganisms Isolated from Vaginal Infections at a Tertiary Hospital in Bangalore, India. Int J Med Students. 2015; 3(1):34-39.
24. Wang JY, Chen PC, Chang SC, Shiau YR, Wang HY, Lai JF, Huang IW, Tan MC, Tsai Lauderdale LY. Antimicrobial susceptibilities of Proteus mirabilis: a longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. BMC Infect Dis. 2014; 14:486, DOI: 10.1186/1471-2334-14-486.
25. Bahashwan SA, El Shafey HM. Antimivcrobial resistance patterns of proteus isolates from clinical specimens. European Sci J. 2013; 9(27):188-202.
26. Adamus-Bialek W, Elzbieta Zajac E, Parniewski P, Kaca W. Comparison of antibiotic resistance patterns in collections of Escherichia coli and Proteus mirabilis uropathogenic strains. Mol Biol Rep. 2013; 40(4):3429-3435.
27. Filgona J, Baneerje T, Anupurba S. Antimicrobial Resistance Pattern of Multidrug Resistant Enterobacteriaceae (MDRE) Isolated from Clinical Samples with Special Reference to Carbapenemase Production and Susceptibility to Tigecycline. British Microbiol Res J. 2014; 4(9):1035-1045.
28. Yusuf I, Arzai AH, Haruna M, Sharif AA, Getso MI. Detection of multi drug resistant bacteria in major hospitals in Kano, North-West, Nigeria Braz J Microbiol. 2014; 45(3):791-798.
29. Senthamarai S, Sivasankari S, Anitha C, Kumudavathi MS, Amshavathani SK, Venugopal V, Thenmozhi Valli P. R.Int J Advances İn Pharm, Biol and Chem. 2015; 4(2):355-360.
30. Feglo PK, Gbedema SY, Quay SNA, Adu-Sarkodie Y, and Opoku-Okrah C. Occurrence, species distribution and antibiotic resistance of Proteus isolates: A case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana. Int J Pharm Sci Res (IJPSR). 2010; 1:347-52.
31. Hameed A, Sadozai SK, Rahim F, Ullah R, Aurakzai R. Study to Determine the Antimicrobial Sensitivity and Resistance pattern of Various Strains against Commonly prescribed Antibiotics. Med. Forum, 2014; 25(7):56-59.
32. Kurtoğlu MG, Bozkurt H, Güdücüoğlu H, Yasemin Bayram Y, Berktaş M. Antimicrobial susceptibilities of Proteus mirabilis strains isolated from clinical specimens. Basic Med J, 2008; 18(1):23-26.
33. Zahid M, Akbar M, Sthanadar AA, Ali PA, *et al*. Isolation and Identification of Multi-Drug Resistant Strains of Non-Lactose Fermenting Bacteria from Clinical Refuses in Major Hospitals of Khyber Pakhtunkhwa, Pakistan. Open J Med Microbiol. 2014; 4:124-131.
34. Alhambra A, Cuadros JA, Cacho J, Gómez-Garcés JL, Alós JI. *In vitro* susceptibility of recent antibiotic-resistant urinary pathogens to ertapenem and 12 other antibiotics. J Antimicrob Chemother. 2004; 53:1090-1094.
35. Khawcharoenporn T, Vasoo S, Singh K. Urinary Tract Infections due to Multidrug-Resistant Enterobacteriaceae: Prevalence and Risk Factors in a Chicago Emergency Department. Hindawi Publishing Corporation Emergency Medicine International, 2013, Article ID 258517, 7 pages.
36. Manisha J, Mitesh HP, Nidhi KS, Dhara JM, Vegad MM. Original Artical Spectrum of Microbial Flora in diabetic foot ulcer and its antibiotic sensitivity pattern in tertiary care hospital in ahmetabad, Gujarat. Nat j Med Res. 2012; 3(2):354-357.
37. Bolaji AS, Akande IO, Iromini FA, Adewoye SO, Opasola OA. Antibiotic resistance pattern of bacteria spp isolated from hospital waste water in Ede South Western, Nigeria. Europ J Experimental Biol. 2011; 1(4):66-71.
38. Jabur MH, Saedi EA, Trad JK. Isolation of Proteus mirabilis and Proteus vulgaris from Different Clinical Sources and Study of some Virulence Factors. J Babylon University/Pure and Applied Sci. 2013; 1(21):1-6.
39. Romanus II, Emmanue NA, Ngozi AF, Onyinyechi UE, Chidiebube NA, Egwu OA, Nnenna NT. Antibiotic susceptibility patterns of bacterial isolates from hospitalized patients in Abakaliki. Int Research J Basic Clinical Studies. 2013; 1(4):46-52.
40. Leulmi Z, Kandouli C, Benlabed K, Lezzar A, Ilhem Mihoubi I. Prevalence and evaluation of resistance to antibiotics of genera Proteus, Morganella and Providencia isolates in University Hospital of Constantine, Algeria. Int J Advanced Res. 2014; 2(1):220-27.
41. Pandey JK, Tyagi AKS. Prevalence of Proteus species in clinical samples, antibiotic sensitivity pattern and ESBL production. Int J Curr Microbiol App Sci. 2013; 2(10):253-61.
42. Tumbarello M, Trecarichi EM, Fiori B, Losito AR, D'Inzeo T, Campana L, *et al*. Multidrug-Resistant

Proteus mirabilis Bloodstream Infections: Risk factors and outcomes. Antimicrob Agents Chemoth. 2012; 56(6):3224-31.
43. Cohen-Nahum K, Saidel-Odes L, Riesenberg K, Schlaeffer F, Borer A.. Urinary tract infections caused by multi-drug resistant Proteus mirabilis: risk factors and clinical outcomes. Infection. 2010; 38:41-46.
44. Steward FS, Beswick TSL. Bactertiology, virology and immunity for students of medicine 10 th Ed. ELBS and Balliere Tindall London. 1977; 235-237.
45. Yah SC, Eghafona NO, Oranusi S, Abouo AM. Widespread plasmid resistance genes among Proteus species in diabetic wounds patients in Abuth Zaria. Afr J Biotechnol. 2007; 6(15):1757-1762.