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General Medicine

PROTEUS MIRABILIS AND URINE IN A TERTIARY CARE CENTRE IN KISHANGANJ, BIHAR.

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ABSTRACT **Aims and objectives:** Urinary tract infection is the commonest infection mainly in female worldwide. Moreover proteus infection is notorious organism because of its biofilm formation under which it may protect itself from the broad spectrum antibiotics and it is resistant to most of the common broad spectrum antibiotics but it will vary in different community. So the aim of this study to demonstrate the spectrum of resistance to different antibiotics by proteus mirabilis in case urinary tract infection in that community so that cautious administration of antibiotic could be done. **Methods:** Urine samples were collected from 1151 patients with complaint related to urinary tract in this tertiary care center and sent for culture and sensitivity. **Results:** Proteus mirabilis were detected in urine samples of 70 patients (males = 25 and females = 45, female: male ratio 1.8:1). As per sensitivity result proteus in this study was highly sensitive to piperacillin-tazobactam (62.85%), imipenem (58.57%), and meropenem (55.71%) only, moderately sensitive to cefoperazone-salbactam (37.14%), ceftriaxone (34.28%), gentamicin (40%), tobramycin (35.71%), Netilmicin (37.14%), amikacin (44.28%), ertapenem (48.57%), levofloxacin (38.57%), highly resistant to amoxycillin (4.2%), cefuroxime (20%), azithromycin (7.14%), aztreonam (14.28%), chloramphenicol (1.42%), polymyxin B (5.71%), colistin (5.71%), ticarcillin (12.85%) and nitrofurantoin (12.85%). **Conclusion:** Antibiotic stewardship and choice of antibiotic are urgently required following the international standard to make policies which will govern the administration of the antibiotics to minimize the resistance in the community or in hospital.

KEYWORDS : Proteus mirabilis, culture and sensitivity, urinary tract infection, tertiary care center.

INTRODUCTION:

Genus proteus was first demonstrated in 1885 by Margit Luise Hauser[1]. It was gram negative ciliated rod shaped organism in the group of Enterobacteriaceae family[2]. Later on this genus has been subdivided into five species, like, proteus mirabilis, proteus vulgaris, proteus hauseri, proteus penneri and proteus myxofuciens and three genomic species, like, proteus genomoespecies 4, 5 and 6[1, 2]. Amongst the all above subgroups, indole, salicin and esculin negative and chloramphenicol resistant species is proteus mirabilis[3]. In the intestinal tract of human being is the residence of many commensal organisms, some of them are opportunistic pathogens as in debilitated immunosuppressed patients they will be responsible for different infections in the body involving skin, joints, digestive tract, joints including brain as a result of bacteremia[4]. Family of Enter obacteriaceae as per report of American centers of disease control 30% of all nosocomial pathogens and 50% of urinary tract pathogens[5]. Proteus is such type of commensal organism resides in the intestine and in opportunistic condition they will spread to produce urinary tract infection[6, 7]. In USA It is responsible for 3% nosocomial infection[7]. Main obstacle to treat this organism is the formation of biofilm produced within 40 hours of intubation, described in 17th century by Antinie van Leeuwenhock who demonstrated bacteria in the dental plaques through optical microscoipe[8, 9, 10]. It is composed of microbacteria itself along with presence of certain surfaces and neighboring cells being covered by extracellular matrix[10, 11]. So any biomaterials. Like, urinary catheter, ureteral stent, vascular catheter, penile implant, prosthetic heart valves develop biofilm[12, 13, 14]. Bacteria living in that biofilm show different behavior as compared to planktonic type, like, susceptibility to antibiotics will be different in both in[12, 15]. This different sensitivity to different antibiotics are due to various mechanisms, firstly, Reduction in distribution of the administered antibiotics into deeper layer of biofilm by mucus and glycocalyx, secondly, changes in their transcription leads to change in activation of genes responsible for resistance to antibiotics, thirdly, enhancement of transfer of genetic information in that organism even in between the species[12, 15].This biofilm producing organism can communicate between with the help of quorum sensing[11,12,15] Again, proteus vulgaris generates ammonia from urea with the help of urease generated by this organism and raise the serum pH above 8.3 of the urine[16]. It also helps in producing crystals of calcium and magnesium phosphates in the urine. Different studies demonstrated different spectrum of resistance to different antibiotics in different areas in India which is a definite threat for treating the patient infected with proteus vulgaris. With respect to this background this present study was performed to demonstrate the spectrum of antibiotic resistance against proteus vulgaris.

MATERIALS AND METHOD:

This cross-sectional observational study of eight months duration was performed in MGM Medical College & LSK Hospital after receiving permission from local ethical committee. Urine samples were collected from 1151 admitted patient having history of burning sensation during micturition with fever with chill and rigor, hesitancy, frequency or urgency in micturition and sent to the laboratory. There urine sample s were inoculated in the sterile Luria Bertani (LB) broth It consists of 10 gm/liter tryptone, 5 gm/liter yeast extract, 0.5 gm/liter sodium chloride as early as possible and incubated in shaking incubator at 37° C for overnight[6]. After overnight incubation it was inoculated in nutrient agar plate. After 48 hours incubation at 37° C isolation was done with the help of CLED (Cysteine lactose electrolyte deficient) media for differentiation of bacterial species[6]. Reference strain of staphylococcus aureus 209P and Escherichia coli 35218 were used found from ATCC (American Type Culture Collection) on 5% sheep blood agar. From this growth isolates were subcultured in fresh agar plate to characterize further along with antimicrobial sensitivity. When inoculates from the LB broth was spreaded in blood and MacConkey agar, this organism demonstrated swarming forming successive waves and formed thin filmy layer in blood agar but not on MacConkey agar. Now inoculates from the LB broth was mixed in Brain-heart infusion broth media containing 20% glycerol and stored at -20° C for demonstration further reaction and bacterial sensitivity. Hemolysis was assessed by suculturing the isolate into sheep blood agar overnight at 37° C and demonstrating the clear zone concentrically around the colonies. Urease activity was assessed according to the protocol of Mobley and Chippendale[17]. A loopful isolates from the broth was spreaded in a clear oil free glass slide and was left for nearly 1 minute after covered with 0.5% crystal violet. After 1 minute Lugol's iodine was poured on the slide and kept it for another 30 seconds. Then this slide was rinsed under water and 1% safranin was poured for two minutes followed by washing it under water and kept it for drying. Now dried slide was seen under phase contrast microscope. Indole test was also done according to standard method of Fingergold et al to detect its positivity[18].

Antibiotic susceptibility pattern: This was done by disk diffusion method as determined by Kirby-Bauer method[19]. Now isolates from the LD broth was spreaded in Mueller-Hinton agar and allowed to grow for 12 hours. Then antibiotics discs impregnated with antibiotics, like, amoxycillin (10 µg), Amoxycillin-clavulanic acid (30 µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (30 µg), Netilmicin (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg), azithromycin (15 µg), polymyxin (300 µg), colistin (100 µg), chloramphenicol (30 µg), cefotaxime (30 µg), cefuroxime (µg),

ceftriaxone (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), piperacillin-tazobactam (30 µg), cefoperazone-salbactam (30 µg).

STATISTICALANALYSIS:'

Data has been analysis by SPSS software version 17.

RESULTS:

Amongst total 1151 urine samples 70 patient suffered from proteus mirabilis related urinary tract infection, females were 45 and males 25 with female to male ratio of 1.8:1. As per sensitivity result proteus in this study was highly sensitive to piperacillin-tazobactam (62.85%), imipenem (58.57%), and meropenem (55.71%) only, moderately sensitive to cefoperazone-salbactam (37.14%), ceftriaxone (34.28%), gentamicin (40%), tobramycin (35.71%), Netilmicin (37.14%), amikacin (44.28%), ertapenem (48.57%), levofloxacin (38.57%), highly resistant to amoxycillin (4.2%), cefuroxime (20%), azithromycin (7.14%), aztreonam (14.28%), chloramphenicol (1.42%), polymyxin B (5.71%), colistin (5.71%), ticarcillin (12.85%) and nitrofurantoin (12.85%).

Antimicrobial Sensitivity pattern of Proteus mirabilis infection urinary tract (n= 70)

Antibiotic	Sensitivity	Percentage
Amoxycillin	3	**4.2**
Amoxycillin-Clavulanic acid	19	27.14
Piperacillin-Tazobactam	44	**62.85**
Cefoperazone-Salbactam	26	**37.14**
Cefuroxime	14	**20**
Cefotaxime	20	28.57
Cefoxitin	11	**15.71**
Ceftazidime	16	22.85
Ceftriaxone	24	**34.28**
Cefepime	16	22.85
Azithromycin	5	**7.14**
Aztreonam	10	**14.28**
Ertapenem	34	**48.57**
Imipenem	41	**58.57**
Meropenem	39	**55,71**
Gentamicin	28	**40**
Tobramycin	25	**35.71**
Netilmicin	26	**37.14**
Amikacin	31	**44.28**
Norfloxacin	16	22.85
Ciprofloxacin	23	**32.85**
Ofloxacin	17	24.28
Levofloxacin	27	**38.57**
Cotrimoxazole	16	22.85
Chloramphenicol	1	**1.42**
Polymyxin B	4	**5.71**
Colistin	4	**5.71**
Ticarcillin	9	**12.85**
Nitrofurantoin	9	**12.85**

DISCUSSION:

Low sensitivity imposes a great threat to the community. According to many studies human intestine is being filled up with bacteria with resistant genes to different antibiotics over several years[20]. So, due to that type of genetic variations during choice of antibiotics the clinicians has to be very particular and should be well concerned about the susceptibility to antibiotics in that community because faulty choice of antibiotics may take the life of the patient. Enterobacteriaceae grow in the intestine of human being which can be separated by indole test, methyl-red, Voges-Proskauer as well as citrate tests to identify different groups, like, Escherichia coli, proteus, enterobacter and klebsiella[21]. Proteus mirabilis is virulent due to presence of different toxins, like, hemolysin, protease, and urease. In the meta-analysis it was shown that Polish strain of proteus strain (collected from Military teaching Hospital No 2, Medical University of Lotz, Poland in 2005 – 2007 and another from Swietokrzyskie Oncology Center in KieIce, Poland in 2002) was resistant to ampicillin (48%), carbenicillin (44%), imipenem (46%), aztreonam (49%), Swedish (Department of Clinical Microbiology of the Karolinska Hospital in Stockholm, Sweden from 1999 October to 2000 January) and laboratory strain (Czech National Collection of type cultures from the Institute of Epidemiology and Microbiology, Prague, Czech Republic stored for 20 years with subcultures for 20 times) was highly sensitive to imipenem, aztreonam and amikacin, but all three strains were highly resistant (90% -- 100%) to tetracycline, polymyxin B, colistin and nitrofurantoin, similarly this present study Proteus Mirabilis demonstrated high resistance to polymyxin B (94.29%), colistin (94.29%), nitrofurantoin (87.15%), aztreonam (85.72%)[23]. but moderately sensitive to all aminoglycosides (35.71% -- 44.28%). Contrary to the behavior of Polish strain this present study showed good sensitivity to imipenem (58.57%) and meropenem (55.71%). This diverse sensitivity in antibiotic sensitivity may be result from frequency and the types of antibiotic use. Again it was shown that in any specific group if one antibiotic was resistant most of the drugs in that group also demonstrated resistance. One study demonstrated resistance of proteus (ESBL positive) to ciprofloxacin was 23.9%, but in case of ESBL negative proteus it was 89.3%[24]. Similar findings (ESBL positive and negative proteus resistant to 14% and 76.9% respectively) also noted in the study of Ho et al[25]. On the contrary in the study by Luzzaro et al. ESBL positive and ESBL negative proteus demonstrated 56% and 2.5% resistance to ciprofloxacin respectively[26]. But this present study demonstrated 76.15% resistance to ciprofloxacin. This present study demonstrated 77.15% resistant to ceftazidime which was contrary to different studies, like, of Cao et al (29.8% resistant), Ko et al (9.1% resistant), Nijssen et al (4.7% resistant), Wang et al (6.7% resistant)[27,28,29,30].

CONCLUSIONS:

From the present study it can be concluded that antibiotic stewardship as well as rational use of proper antibiotics usually sensitive to this organism in that community will minimize the chance of resistance. Antibiotic choice should follow the antibiogram existent in that community or hospital. So it requires some policies in that hospital consistent with the international standards of stewardship in antibiotic, which determines "the use or not to use frequently" antibiotics.

REFERENCES:

1. Rozalski A, Kwil I, Torzewska A, Baranowska M, Stacrek. "Proteus bacilli: features and virulent factors,"Postppy Higieny i Medycyny Doswiadczalnej. 2007;61:204-219.
2. O'Hara CM, Brenner FW, Miller JM. "Classification, identification and clinical –lactamases,"significance of Proteus, Providencia and Morganella". Clinical Micribiology Reviews. 2000;13(4):534-546.
3. Hickman FW, Steigerwalt AG, Farmer III JJ, Brenner DJ. Identification of proteus penneri sp. Nov., formerly known as proteus vulgaris indole negative or Proteus vulgaris biogroup. J Clin. Microbiol. 1982;15:1097-1102.
4. Endimiani A, Luzzaro F, Brigante et al. Proteus mirabilis bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum β-lactamases," Antimicrobial Agents and chemotherapy. 2005;49(7):2598-2605.
5. Jamil M, Bashir S, Mohsin M, Tariq A, Bashir A, Haque A et al. Differentiation of common Gram Negative Pathogens by PCR-Ribotyping. Pak J Med. Sci. 2007;23:233-237.
6. Dash SK, Chakraborty SP, Mandal D, Roy S. Isolation and Characterization of multidrug resistant uropathogenic Escherichia coli from urine sample of urinary tract infected patients. International Journal of life Science & Pharma Research. 2012;2:2250-0480.
7. Stamm WE, Hooton TM. The management of urinary tract infection. N Engl J Med. 1993;329:1328-1334.
8. Jacobsen SM, Shirtliff ME. Proteus mirabilis biofilms and catheter-associated urinary tract infections. Virulence. 2011;2(5):460-465.
9. Wasfi R, AbdEl-Rahman OA, Mansour LE, Hanora AS, Hashem AM, Ashour MS. Antimicrobial activities against biofilm formed by proteus mirabilis isolates from wound and urinary tract infection. Indian J of Med Microbiol. 2012;30(1):76-80.
10. Donlan RM. Biofilm formation: a clinically relevant microbiological process. Clinical Infectious Diseases. 2001;33:1387-1392.
11. Karatan E, Watnick P. Signals, regulatory networks and materials that build and break bacterial biofilms. Microbiol and Molecular Biol Reviews. 2009;73(2):310-347.
12. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. International J of Antimicrobial Agents. 2010;35(4):322-332.
13. Hoiby N, Ciofu O, Johansen HK et al. The clinical impact of bacterial biofilms. International J of oral Science. 2011;3(2):55-65.
14. Whitfield H, Choong S. Biofilms and their role in infections in urology. BJU International. 2000;86(8):935-941.
15. Stewart PS. Mechanism of antibiotic resistance in bacterial biofilms. International J of Med Microbiology. 2002;292(2):107-113.
16. Stickler D, Morris N, Moreno MC, Sabbuba N. Studies on the formation of crystalline bacterial biofilms on urethral catheters. Eur J Clin Microbiol Infect Dis. 1998;17(9):649-652.
17. Mobley HL, Chippendale GR. Hemagglutinin, urease and hemolysin production by Proteus Mirabilis from clinical sources. J Infect Dis. 1990;161(3):525-530.
18. Fingergold SM, Sutter VL, Ahebery HR, Rosenblatt JE. Isolation of anaerobic bacteria. Man Clin Micr Biol. 1974;365-375.
19. Bauer AW, Kirby WMM, Sherris JC, Turch M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol.1966;45:493-496.
20. Okeke IN, Susan T, Fayinka, Adebayo L. Antibiotic resistance in Escherichia coli from Nigerian students. 1986-1998. Emerging Infectious Diseases. 2000;6(4).
21. Prescott LM, Harley JP, Klein DA. Isolation of pure bacterial cultures from specimens. Microbiology International (4th edition). WCB McGraw's Hill Companies Boston. 1999;714-796.
22. Santo E, Salvador MM, Marin JM. Multidrug-resistant urinary tract isolates of Escherichia coli from Ribeirao Preto, Sao Paulo, Brazil. The Brazilian Journal of Infectious Diseases. 2007;11:575-578.
23. Adamus-Bialek W, 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;40:3429-3435.
24. Kanayama A, Iyoda T, Matsuzaki K et al. Rapidly spreading CTX-M-type β–lactamase –producing Proteus Mirabilis in Japan. International J of Antimicrobial Agents. 2010;36(4):340-342.
25. Ho PL, Ho AYM, Chow KH et al. Occurance and molecular analysis of extended-spectrum β-lactamase-producing Proteus mirabilis in Hong Kong, 1999-2002. J of Antimicrobial Chemotherapy. 2005;55(6):840-845.
26. Luzzaro F, Perlli M, Amicosante et al. Properties of multidrug-resistant, ESBL-producing Proteus Mirabilis isolates and possible role of β-lactam / β-lactamase inhibitor combinations. International J of Antimicrobial Agents. 2001;17(2):131-135.
27. Cao V, Lambert T, Nhu DQ et al. Distribution of extended spectrum β-lactamases in clinical isolates of Enterobacteriaceae in Vietnam. Antimicrobial agents and chemotherapy. 2002;46(12):3739-3743.
28. Ko KS, Lee MY, Song JH et al. Prevalence and characterization of extended-spectrum β-lactamase-producing Enterobacteriaceae isolated from Korean hospitals. Diagnostic Microbiology and Infectious Disease. 2008;61(4):453-459.
29. Nijssen S, Florijn A, Bonten JM, Schmitz FJ, Verhoef J, Fluit AC. Beta-lactam susceptibilities and prevalence of ESBL-producing isolates more than 5000 European Enterobacteriaceae isolates. International J of Anitimicrobial Agents. 2004;24(6):585-591.
30. Wang H, Chen M, Ni Y et al. Antimicrobial resistance among clinical isolates from the Chinese Meropenem surveillance study (CMSS), 2003-2008. International J of Antimicrobial agents. 2010;35(3):227-234.