Isolation, Characterization And Antibiogram Of *Proteus* Species In The Urine Of Male Students Of University Of Abuja, Gwagwalada, Abuja

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Abstract: The isolation, characterization and antibiogram of *Proteus* species in the urine of male students of University of Abuja was carried out. One hundred and fifty four (154) early morning mid-stream urine samples were collected from the male students. Ten-fold serial dilutions of the samples were carried out. 0.2ml of 10^{-3} dilutions of each urine sample was aseptically inoculated onto freshly prepared Cystine Lactose Electrolyte-Deficient (CLED), Nutrient agar and MacConkay agar plates using the spread plate technique and incubated for 24 hours at 37^0C. The resulting colonies were counted and further identified by cultural, morphological and biochemical characteristics. The antibiotic susceptibility tests were carried out on the isolated *Proteus* species using standard antibiotic discs. The results obtained showed that the mean total *Proteus* count ranged from $1.21x10^2 \pm 1.0 - 1.01x10^3 \pm 7.1$ cfu/ml. Out of the 154 samples examined, 151 (98.10%) were positive for bacteriuria. Furthermore, it was also observed that 10 (6.5%) samples were positive for *Proteus* species infection. The results show that the Proteus species isolated in this work were susceptible to Ciprofloxacin, Gentamicin, Pefloxacin and Sparfloxacin and as such are suitable for the treatment of urinary tract infections caused by *Proteus* species.
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1.0 Introduction

Urinary tract consists of the various organs that produce, store and get rid of urine and it include the kidneys, the ureters, the bladder and the urethra (Vinay and Reddy, 2008). The kidneys filter blood and the filtrate is processed to separate out waste products and the excess amount of minerals, sugar and other chemical. The waste products of metabolism enter the urine where it flows through ureters into the bladder and held until the body is ready to get rid of it. When one urinate, muscles in the bladder wall help push urine out of the bladder through the urethra, and finally out of the body (in males, the urethra passes through the penis while in women, the urethra open just in front of the vagina). Urine (latin "Urina") is a typically sterile liquid by-product of the body secreted by the kidneys through the process called urination, and excreted through the urethra (Kenneth, 2008).

Urinary Tract Infection (UTI) is the most common clinical manifestation of *Proteus* infection. *Proteus* infection accounts for about 1 - 2% of UTIs in healthy women and 5% of hospital acquired UTIs. Complicated UTIs (i.e. those associated with catheterization) have a prevalence of 20 - 45% (Engel and Schaeffer, 1998).The urinary tract can be infected from above (by bacteria entering the kidneys from the blood stream and travelling downward) or from below by bacteria entering the urethra and travelling upward (Zahoor *et al*., 2005). Infection results when the bacterial virulence factor overcomesthe numerous host defense mechanisms. In most instances, growth of more than 10^5 organisms per milliliter from a properly collected midstream urine sample indicates urinary tract infection.

However, the aim of this study is to isolate and characterize *Proteus* species found in the urinary tract of male students in the University of Abuja and as well as ascertain the antibiogram pattern of the *Proteus* isolates to antibiotics using the antibiotics disc.

2.0 Materials and Methods

2.1 Study Area

Gwagwalada is located in Abuja, the Federal Capital Territory of Nigeria. It lies between latitude 8^0 – 9^0N and between longitude 7^0 – 8^0E, with a total land area of 1, 043km^2 and annual humidity of 20 - 30%, and average temperature of between 27^0C – 32^0C. It has an estimated population of 157, 770 at the 2006 census.

2.2 Study Population

One hundred and fifty four early morning mid stream urine samples were collected randomly from male students of the University of Abuja who volunteered to be included in the study. The sampling was carried out between August and October 2012.

2.3 Media Preparation

The media used in this work include Cystine Lactose Electrolyte Deficient (C.L.E.D) agar, Nutrient agar and MacConkay agar, all of MercKGaADarstadt brand, Germany. The media were prepared based on manufacturer's instructions and sterilized by autoclaving for 15 minutes at 121^0C.

2.4 Sample Collection

Early morning mid-stream "clean catch" urine samples were collected from students, using universal sterile containers with screw caps. The individuals were instructed on how to collect the samples observing all aseptic conditions such as washing the penis with sterile water or with mild alcohol and avoiding the penis from making contact with the container. After collection, the samples were transported immediately to the laboratory for analyses.

2.5 Microbiological analyses of urine samples

This was carried out according to the method described by Chessbrough (2006).Ten-fold serial dilutions of the urine samples were carried out, and 10^{-3} dilution of each urine sample was inoculated onto C.L.E.D agar and Nutrient agar plates, using the spread plate technique. This was done by using pipette to transfer 0.2ml of 10^{-3} dilution onto two different agar plates containing C.L.E.D agar and nutrient agar. A sterile hockey stick- shaped glass rod was used to spread the sample evenly on the agar plates. These plates were left on the bench for about 15 minutes, before they were incubated for 24 hours at 37^0C. After incubation, colonies on both plates were counted as colony forming units (cfu/ml). The average colony obtained was multiplied by the dilution factor and divided by 0.2 ml to give an estimated number of bacteria present per milliliter of each urine sample. Representatives of each characteristic and discrete type of colonies on the agar plates were further purified to obtain pure cultures by sub culturing onto a selective medium, MacConkey Agar media. The plates were then incubated at 37^0C for 24 hours.

2.6 Characterization of Isolates

Isolates from pure culture were characterized on the basis of colonial morphology, microscopy and biochemical reactions as follows:

2.6.1 Gram Staining

According to Chessbrough (2006), a thin smear of each culture was made, air dried and heat fixed. Each smear was flooded with crystal violet and allowed to stay for 60 seconds before washing with water. Gram's iodine was added to each slide and was allowed to remain for 60 seconds and washed with water. The iodine solution acted as a mordant. Each slide was slightly tilted and 95% ethyl alcohol was gently used to cover the smear on the slide so that it runs off the edge of the slide and was rinsed with water. The slides were counter stained with safranin for 30 seconds and then rinsed with water. The slides were air-dried and viewed using the ×10 objective.

2.6.2 Urease Test

This was done according to Cowan and Steel (2002). Urease agar was prepared according to manufacturer's directives, in bijou bottles. The test organisms were then inoculated on the slant surface and then incubated at 37^0C for 12 hours. The production of a pinkish-red color on the medium indicates a positive result. Whereas if the color of the medium remains unchanged (pale yellow-pink), the result is negative.

2.6.3 Citrate Utilization Test

Slopes of citrate agar were prepared in bijou bottles as recommended by the manufacturer. With a sterile wire loop, the slopes were streaked with a saline suspension of the test organisms, and incubated at 37^0C aerobically for 48 hours. Bright blue was indicative of positive test result.

2.6.4 Indole Production Test

The test organism was inoculated in a bijou bottle containing 3ml of sterile tryptone water. This was then incubated at 37^0C for up to 48 hours. Indole was then tested for by adding 0.5ml or about five drops of Kovac's reagent. The bottle was shaken gently and examined for a red color on the surface layer within 5 - 10 minutes. Red surface layer was an indication of positive result.

2.6.5 Triple Sugar Iron Test

A sterilized inoculation needle was used to pick a colony of the test organism. This was used to stab the butt and rub the slant of the agar contained in a test tube. The inoculated test tubes were incubated for 24 hour at 37^0C before checking for sugar fermentation and gas production.

2.7 Antibiotics Susceptibility Testing

Antibiotics susceptibility tests of the *Proteus* isolates was determined using the modified Kirby-Bauer method (Disc diffusion method). Maxi Discs (Nigeria) produced the standard antibiotics multi discs used, for gram-negative organism since *Proteus* is gram-negative bacteria. The multi discs composed of Septrin (30µm), Chloramphenicol (30µm), Sparfloxacin (10µm), Tetracycline (25µm) Ciprofloxacin (10µm), Gentamicin (10µm), Pefloxacin (30µm), Amoxicillin (30µm), Tarivid (10µm) and Streptomycin (30µm).

According to Bauer *et al*. (1996), a colony of each of the test organisms are emulsified in nutrient broth. Sterile swab was used to inoculate the bacterial suspension on the surface of the solid nutrient agar plates evenly. The plates were allowed to stay for 5 minutes on the work bench and a sterile forceps was used to place the antibiotics discs on the centre of the inoculated plates aseptically. The plates were allowed to stand for 30 minutes to enable the antibiotics to diffuse into the media properly. The plates were then incubated at 37^0C for 18 hours.

The diameter of the zones of inhibition produced by each antibiotics on the discs were measured using a transparent ruler and the result recorded in millimeters and interpreted as either susceptible (S) or resistance (R) to the antibiotic agent used, depending on the zone diameter of inhibition produced in comparison with reported standard length: 0-5 mm regarded as resistant (R), 5-15 mm regarded as sensitive (S_1) , 15-25 mm (S_{11}) and 25-35 mm (S_{111}).

2.8 Statistical Analysis

The data were subjected to chi-square test and significance was accepted at $P \leq 0.05$ level of significance.

3.0 Result

Table 1 shows the overall percentage rate of bacteriuria of the urine sample of male students of University of Abuja from August to October 2012. Out of the 154 samples examined, 151 (98.10%) were positive for bacterial infection. Furthermore, out of the 154 samples examined, 10(6.5%) were contaminated with *Proteus* species.

Table 1: Percentage Rate of *Proteus* species Contamination of the Urine samples

Number of samples examined	Total Number of samples Contaminated by Bacteria	Total Number of samples Contaminated by *Proteus* spp
154	151 (98.10%)	10 (6.5%)

Table 2 shows the mean total *Proteus* count (cfu/ml) of the urine samples of male students of University of Abuja, collected from August to October 2012. The mean total *Proteus* count ranged from 1.21 x $10^2\pm$ 1.0 – 1.01 x $10^3\pm$ 7.1 cfu/ml with a grand mean total *proteus* count of 5.84 x $10^3\pm$ 7.2.

Table 2: Total *Proteus* Count of the Urine Samples of Male Students of University of Abuja, Abuja

S/no	Mean Total *Proteus* Count (cfu/ml)
1.	4.95 x $10^2\pm$5.0
2.	6.15 x $10^2\pm$5.0
3.	8.25 x $10^2\pm$5.0
4.	1.01 x $10^3\pm$ 7.0
5.	6.95 x $10^2\pm$ 5.0
6.	5.05 x $10^2\pm$ 15.0
7.	9.05 x $10^2\pm$ 5.0
8.	4.85 x $10^2\pm$ 5.0
9.	1.21 x $10^2\pm$ 1.0
10.	1.85 x $10^2\pm$ 15.0
Grand mean	5.84 x $10^3\pm$ 7.2

Note: Values are expressed as mean of the duplicates ± Standard Deviation

Table 3 shows the morphological characterization of isolates. The species, of *Proteus* were grouped into two based on Indole test: indole positive (probable species: *Proteus vulgaris*) and indole negative (probable species: *Proteus mirabilis).* All the species were urease, citrate, H_2S positive and produce gas on TSI agar and as such are gram negative. While only two (20%) of the species were Indole positive and 8(80%) were Indole negative. Both groups produced yellow butts and red slants on TSI agar slant.

Table 3: Morphological and Biochemical characterization of the urine isolates

Characterization	Isolate 1	Isolate 2
Colonial morphology		
Color	Blue	Blue
Optical characteristics	Translucent	Translucent
Margin	Irregular	Irregular
Elevation	Slightly elevated	Slightly elevated
Size	Small	Small
Biochemical characterization		
Urease test	+	+
Indole test	+	-
Citrate test	+	+
H_2S	+	+
Triple sugar iron test		
G	+	+
Butt	Y	Y
Slant	R	R
Gram's reaction	-	-
Probable organisms	*Proteus vulgaris*	*Proteus mirabilis*

Key:
G=Gas, Y= Yellow, R=Red, + = positive, - = negative

Table 4 shows the antibiogram results of *Proteus* isolates among the ten different antibiotics used namely: Septrin, Chloramphenicol, Sparfloxacin, Tetracycline, Ciprofloxacin, Gentamycin, Pefloxacin, Amoxacillin, Tarivid and Streptomycin. The isolates were moderately sensitive to Tarivid. *P.mirabilis* was sensitive to Chloramphenicol while *P. vulgaris* shows complete resistance to Chloramphenicol. In addition, the isolates were intermediately resistant to Amoxicillin and were very resistant to Streptomycin, Septrin and Tetracycline.

Table 4: Antibiogram of some antibiotics against *Proteus* species isolated from the Urine Samples

	Zone diameter of Inhibition (mm)									
Isolates	**OXF**	**CPX**	**CH**	**PEF**	**SP**	**AM**	**TET**	**GN**	**S**	**SXT**
P. vulgaris	5^R	20^M	0^R	20^M	15^M	2^R	0^R	15^M	0^R	0^R
P. mirabilis	10^L	26^H	8^L	22^M	16^M	5^R	0^R	19^M	0^R	0^R

Key:
SXT: Septrin, CH: Chloramphenicol, Sp: Sparfloxacin, CPX: Ciprofloxacin, GN: TET: Tetracycline, Gentamycin, Pef: Pefloxacin, AM: Amoxacillin, OFX: Tarivid and S: Streptomycin
Key: R=Resistant, L = low sensitive, M = moderate sensitive, H= high sensitivity.

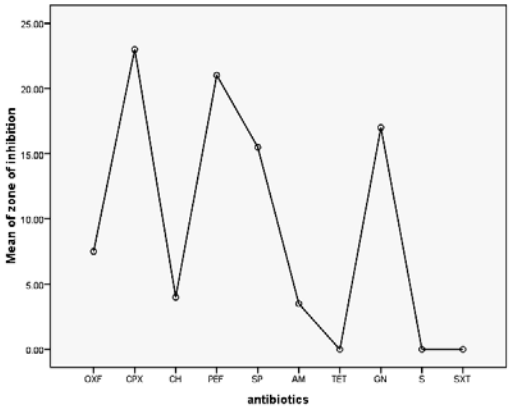

Figure1: Mean Zone diameter of Inhibition of the Antibiotics against the *Proteus* species isolated from urine samples

4.0 Discussion

In this study, out of the 154 samples collected, 10(6.5%) samples gave positive and significant *Proteus* counts, 141 samples gave other various bacterial growth while 3 samples gave no growth. A single colony of *Proteus* species in the urine is of health significance since the urine is supposed to be sterile. Also, owing to the fact that *Proteus* is not a normal flora of the urine; its presence is a sign that the urine is contaminated.The result of the incidence of *Proteus* species in this study was almost similar to that obtained by Shah *et al.* (2002), where as low as 6.67% was reported, but less than the results from the works of some other scientists such as that reported by Nicolas *et al*., (1997). However, the result of this work shows that the incidence of *Proteus* induced urinary tract infection among the male students of University of Abuja is relatively low (6.5%) compared to that

from other places. This low incidence or prevalence could be because of the age grade from which the urine samples were collected (as the samples were mainly collected from student within the age of 18-32). This is in accordance with the findings of Shah *et al*. (2002), where the incidence of *Proteus* induced urinary tract infection increased with age as evident by the highest incidence occurring at the age of 50-60yrs. So also, Senior (1977), reported that urinary tract infection with *Proteus*, were frequently only in patients of 60 years and above. Sex is another factor that can be considered as the reason for the low incidence of *Proteus* in this study, since females are believed to be more infected with UTI than males except at the extreme of life (Akinkugbe *et al*., 1973). This is because of the shorter and wider urethra in females (Duerden *et al*., 1990). Another reason can be drawn from the fact the samples were collected from non-hospitalized patients and patients without in-dwelling catheters (Karen *et al*., 1994).

The sensitivity pattern for *Proteus* in this study shows that Ciprofloxacin was the most effective drug for *Proteus* induced urinary tract infection among the antibiotics used. It was followed by Pefloxacin, Gentamicin and Sparfloxacin. Tarivid was moderate. *P. mirabilis* was sensitive to chloramphenicol while *P. vulgaris* was completely resistant to Chloramphenicol. The isolates were moderately resistant to amoxicillin and were very resistant to Streptomycin, Septrin and Tetracycline. The sensitivity pattern of *Proteus* species to antibiotics, reported by other works showed similar result except in few cases. According to the work of Theodore (2007), 100%, 85.7% and 14.3% effectiveness were reported for Ciprofloxacin, Gentamycin and Chloramphenicol respectively. In this study, it was discovered that *P.vulgaris* was resistant to Chloramphenicol while *P. mirabilis* was sensitive to the antibiotics. This is in agreement with the findings of Yah *et al*. (2007) and Enabulele *et al*. (2006). Also, according to the work carried out by Mordi and Momoh (2008) on the incidence of *Proteus* species in wound infections and their sensitivity pattern,100%, 85%, 100% 28%, 0% and 0% efficacy were reported for Ciprofloxacin, Gentamycin, Ofloxacin (Tarivid), Chloramphenicol, Streptomycin and Tetracycline respectively. According to literature, *Proteus* species are susceptible to ureido-penicilline (eg Amoxicillin) (Krajden *et al*., 1984). However, in this study, the isolates showed complete resistance to the antibiotics. In addition, the isolates (*Proteus* species) were very resistant to Septrin (0%). These observations can be explained based on the wide spread plasmid resistant genes among *Proteus* species as reported by Enabulele *et al*. (2006).

Conclusion

This study has established that the prevalence of *Proteus* species in the urinary tract of male students of University of Abuja is low (6.5%).This low prevalence could be because the urine samples were collected from male students only, since the prevalence of UTIs is higher in females. This could also be as a result that the samples were collected from non-hospitalized and not catheterized patients. The antibiotics susceptibility test demonstrated that *Proteus* species have a wide range of resistance to several antibiotics and hence the emergences of resistant strains. This could be due to the abuse and misuse of antibiotics. Ciprofloxacin showed the highest susceptibility against the isolates. Therefore, it is the most effective drug while Septrin, Streptomycin and Tetracycline were the least susceptible drugs.

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REFERENCES

1. Akinkugbe, F.M., Familusi, F.B. and Akinkube, O. (1973). Urinary Tract Infection in Infancy and Early Childhood. *East Africa Medical Journal*, 50(9): 514-522.
2. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Jurck, M. (1996). Antibiotics Susceptibility by a Standard Disc Method. *American Journal of Clinical Pathology*, 451:493-496.
3. Chessbrough, M. (2006). *District Laboratory Practice in Tropical Countries*. Part 2: Cambridge University Press United Kingdom. Pp: 135-162.
4. Cowan, S. T. and Steel, K.J. (2002). *Manual for the Identification of Medical Bacteria* (2nd Ed): Cambridge University Press, London. Pp: 205-209.
5. Duerden, B.I., Reil, T.M.S. and Turk, D.C. (1990). A New Type of Medical Parasitic Infection. *American Journal of Infectious Diseases*, Cambridge University Press, London. (3): 576-581.
6. Enabulele, L.O., Yah, S.C., Yusuf, E.O. and Eghafona, N.O. (2006). Emerging Quinolone Resistant Genes among Gram-negative Bacteria Isolated from the Faeces of HIV/AIDS Patients Attending Clinics and Hospital in Benin. *African Journal of Biotechnology*, 8(5): 725-730.
7. Engel, J.D. and Shaeffer, A.J. (1998). Evaluation of Antimicrobial Therapy for Recurrent Urinary

Tract Infection in Women. *Urology of Clinics in North America*, 25(4): 685-701.
8. Karen, C., Deron, C.H., Donal, H.V., Clenn, C.R., Leslie, T.H. and John, M.M. (1994). Laboratory Evaluation of Urinary Tract Infection in an Ambulatory Clinic. *Journal of American Clinical Pathology*, 101.100-103.
9. Kenneth, A.R. (2008). *Bacteriology* (2nd Ed). Cambridge University Press, London. Pp: 56-60.
10. Krajden, S., Fuksa, M., Barton, L. and Lee, A. (1984). *Proteus penneri* and Urinary Calculi Formation. *Journal* of Clinical Microbiology, 19: 541-542.
11. Mordi, R.M. and Momoh, M.I. (2008). Incidence of *Proteus* Species in Wound Infections and their Sensitivity Pattern in the University of Benin Teaching Hospital. *African Journal of Biotechnology*, 8(5): 725-730.
12. Nicolas, M.H., Chardon, H., Aurie, J.L., Cattoen, Y., Croixe, J.C. and Dabernat, H. (1997). Susceptibility of Enterobacteriaceae to Betalactams and Fluoroquinolones. A French multicenter study. *Journal of Clinical Microbiology and Infection*, 3(2): 338-74.
13. Shah, A.A. F., Hassan, I. and Hameed, W. (2002). Prevalence of Urinary Tract Infection in Quaid-L-Azan University, Islambad, Pakistan. *Journal of Infectious Disease*, 7(2); 28-9.
14. Senior, W. (1977). Typing of *Proteus* Strains by Proticine Production and Sensitivity. *Journal of Biotechnology*, 10(7): 7-8.
15. Theodore, M. (2007). Prevalence and Antibiogram of Urinary Tract Infection among Prison Inmates in Nigeria. *The Internet Journal of Microbiology*, 1: 166-169.
16. Vinay, N. and Reddy, M.D. (2008). Urinary Tract, Kidney and Bladder Infections. *Infectious Diseases*, 1:10-12.
17. Yah, S.C., Eghafona, N.O. and Abaoa, A.M. (2007). Wide Spread Plasmid Resistant Genes among *Proteus* Species in Diabetic Wound Patients in ABUTH, Zaria. *African Journal of Biotechnology*, 6(15):1757-1762.
18. Zahoor, A., Hassan, K., Tasleem, A. Z. and Jeved, A. (2005). Urinary Tract Infection: An Old Disease in the New Age. *Journal of Urinary Traction Infection*, 2(12):166-169.

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