Isolation and Identification of Non-gonococcal Organisms Associated with Urethritis from Clinical Samples.

Adomi, P O.

Abstract

Introduction: Urethritis is an inflammation of the urethra, the tube that carries urine from the bladder to the outside of the human body. Many of the Infectious causes of urethritis are sexually transmitted and categorized into gonococcal urethritis (GU) i.e due to infections with *Neisseria gonorrhoeae* and non-gonococcal urethritis (NGU) due to organisms other than gonorrhoeae. The main purpose of study was to isolate and identify microbial agent of urethritis.

Material and Methods: Cultural and Biochemical methods were used to isolate and identify microbial agents of urethritis from urine and urethra collected randomly from 200 patients with clinical urethritis.

Results: Non gonococcal agents of urethritis were isolated, they include the following microorganisms *Escherichia coli* (31.6%) *Staphylococcus aureus* (19.5%), *Candida albicans* (15.0%), *Proteus mirabilis* (13.5%), *Pseudomonas aeruginosa* (7.5%), *Staphylococcus saprophyticus* (7.5%) and *Klebsiella pneumoniae* (5.3%).*Pseudomonas aeruginosa* have not been mentioned previously as agent of non gonococcal urethritis.

Conclusion: Agents of nongonococcal urethritis were isolated. These include three gram negative rods, two gram positive cocci, and a fungus.

Keywords: Nongonococcal urethritis, bacterial, fungi, cultural, biochemical, identification.

Department of Microbiology, Faculty of Sciences, Delta State University Abraka, Nigeria.
Email: padomi.adomi07@gmail.com

Correspondence: Adomi PO, Department of Microbiology, Faculty of Sciences, Delta State University Abraka, Nigeria.

Introduction

Urethritis is a sexually transmitted disease which occur in both male and female, however, urethritis is under diagnosed in females. Urethritis is an inflammation of the urethra, the tube that carries urine from the bladder out of the human body. Many infectious causes of urethritis are sexually transmitted and categorized into gonococcal urethritis (GU) i.e due to infections with *Neisseria gonorrhoeae* and non-gonococcal urethritis (NGU) due to cases other than gonorrhoea[1]. Urethritis can also be classified into specific urethritis if caused by infections transferred through sex (ITTS) by gonococcus, virus of herpes simplex, mycoplasma and unspecific urethritis if caused by conditionally pathogenic microflora: streptococci, staphylococci, coliform bacteria, *Proteus* and fungi.

Urethritis occur worldwide, with approximately 62 million new cases of gonococcal urethritis and

89 million nongonococcal urethritis cases reported yearly[2].

Nongonococcal urethritis (NGU) is among the most widespread conditions for which care is provided in the western world and is the most commonly diagnosed in men attending sexually transmitted disease clinics[3]. In 1972, gonorrhoeae was surpassed by NGU as the more common diagnosis made at private physician offices. Since then the divergence between the two has progressively increased [4].

The morbidity associated with NGU are known to be approximately equal in severity to those of gonococcal disease. However, in contrast to gonorrhoeae, the infectious agents that causes NGU are non-reportable, with the exception of *Chlamydia trachomatis*. The sexual fitness of NGU patients are mostly undetected, accounting for the relative rising incidence of NGU with respect to gonococcal diseases [5]. The prevalence rates of sexually transmitted infectious (STI) present major health, social and economic problems in the developing world leading to morbidity, mortality and stigma [6]. The prevalence rate of STI are far higher in developing countries this is because STI treatment is less accessible, the disease management is through syndromic approach thus asymptomatic cases go undetected and untreated.

Morbidity due to urethritis occur both in men and women. About 1-2% of male patients with urethritis develop urethra stricture or stenosis. Other potential complications include post acute epididymitis, abscess formation, proctitis and infertility, sexually acquired reactive arthritis (Reiter syndrome)[7]. Pelvic inflammatory disease may complicate the disease in females. This disease may subsequently cause infertility, and ectopic pregnancy because of post inflammatory scar formation in the fallopian tubes. Children born to mother infected with chlamydia may develop conjunctivitis, otitis media or pneumonia if exposed to the organs passing through the birth canal. Disseminated syndrome occur in fewer female patients[7]. The purpose of this study was to isolate and identify microbial agents of urethritis as a away of gaining deeper understanding into the pathogenesis and hence the management of the disease.

Materials And Methods

Study design; setting and Population

This was a cross-sectional study that was conducted at General hospital Warri, Eku Baptist hospital and Delta State University Health Centre Abraka all in Delta State Nigeria between May, 2010 and January, 2011.

The study population consisted of adult male and female patients who have been diagnosed clinically with urethritis based on history and presentation with any of the following symptoms: dysuria, urgency, frequency, hesitancy, urethral discharge, dyspareunia and abnormal vaginal discharge. Samples were drawn from population of patients who presented with urethritis symptoms. Samples were collected randomly from both males and females within the sampling period. Samples were collected until the total of 200 was achieved.

Sample Collection and Culture

Clinical specimens from urine and urethral swabs were collected by methods described by Cheesebrough, (2004)[8] Clean catch midstream urine samples were collected in sterile universal containers, while specimen from urethra was collected by gently inserting about 2 centimetres of sterile cotton swab into the urethra and turned. A total of 200 specimens obtained from patients were labelled and

subjected to microbiological analysis, using standard streaked plate method as previously prescribed by Cowan and Steel, (1993)[9]. Specimens from urethral swab, and urine were inoculated onto Nutrient agar, MacConkey agar, Cystine lactose electrolyte –deficient agar and Chocolate agar. The urine sediments were inoculated into Saboraud dextrose agar (SDA) [10,11]. Plates were incubated at 37°C for 24 hours , Chocolate agar plates were incubated in carbon (iv)oxide using candle extinction Jar. While SDA plates were incubated for 25 °C and 37°C for 24-72 hours. After the period of incubation, the plates having significant growth were subcultured until pure colonies were obtained. The pure cultures were preserved in agar slants for further study.

Statistical analysis was simple frequency table using Excel spread sheet.

Identification

Cultures were identified using standard biochemical methods, Cultural characteristics in selective media, Gram staining and biochemical reactions, according to Bergeys Manual of systemic bacteriology [12]. Biochemical test used to confirm the various bacterial isolates were Coagulase test, Catalase test, Indole production, Methyl red test, Voges-proskauer reaction, Urease production, Citrate utilization, sugar fermentation and Haemolysis test on blood agar plates [8].Yeast was identified using standard methods such as Gram staining, germ tube formation, chlamydospores formation on corn meal agar and sugar fermentations and assimilation.

Ethical Clearance

Ethical clearance to conduct this study was obtained from the health institutions used for study. Samples were drawn from population of patients who presented with urethritis symptoms.Samples were collected randomly from both males and females within the sampling period. Samples were collected until the total of 200 was achieved. The cost of the procedure was borne by the researcher.

Results

The demographic information of patients as obtained from Laboratory record is presented in Table 1. Table 1 shows that 60(30%)were in the age group of 26-30years. Males 118 (59%) than females 82 (41%) of which 114 (57%) were married.

Bacterial and fungal isolates obtained from cases of Urethritis.

Of the 200 samples collected, 133 were positive for at least one organsm. Table 2 shows microorganisms isolated from patients with urethritis at the study location. These organisms were *Escherichia coli*, *Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella pneumoniae Proteus mirabilis, Pseudomonas aeruginosa, and Candida albicans* and Table 3 shows cultural and biochemical characteristics of microorganism isolated from various samples. *E. coli*. isolates (31.6%) were confirmed as positive for catalase test, indole production, methyl red test, production of acid from glucose, manitol, lactose, and sucrose but negative for urease production, citrate utilization and Voges-Proskauer reaction [12]. *Staphylococcus aureus*, (19.5%) were found as positive for coagulase, catalase, methyl red, Voges-Proskauer, heamoglobin test and negative for oxidase and indole tests. They also produced acid from glucose, lactose and sucrose. According to Holt *et al.*, 1993, the isolates appeared to be *S. aureus.*

Klebsiella pneumoniae (5.3%) found as negative for Voges proskauer, and positive for lactose, urease, citrate and negative for hydrogen sulphide. *Proteus mirabilis (*13.5%) did not ferment lactose,

positive for urease, indole, motility and produce hydrogen sulphide. *Pseudomonas aeruginosa* (7.5%) were found as positive for oxidase enzyme, produce acid from glucose but no gas production. *Staphylococcus saprophyticus* (7.5%) was positive for catalase, negative for coagulase and haemolysis.*Candida albicans* (15%) was gram positive,produce germtube , pseudohyphae and fermented various sugars.

Table 1 :the Demographic Characteristics of Patients

CHARACTERISTICS	**FREQUENCY (N=200)**	**PERCENTAGE (%)**
Age group (years)		
20-25	50	25
26-30	60	30
36-40	24	12
41-45	12	6
46-50	20	10
51-55	12	6
56-60	4	2
Sex		
Males	118	59
Females	82	41
Marital status		
Married	114	57
Single	86	43

Table 2: Percentage of microorganism isolated from clinical specimens.

Isolates	**No N=133**	**Percentage%**
Escherichia coli	42	31.6
Staphylococcus aureus	26	19.5
Candida albicans	20	15.0
Proteus mirabilis	18	13.5
Pseudomonas aeruginosa	10	7.5
Staphylococcus saprophyticus	10	7.5
Klebsiella pneumonia	7	5.3

Table 3: Cultural and Biochemical characteristics of isolates

									Acid from sugars				Triple sugar from medium							
Isolates	Gram stain	Indole production	Methyl red test	Voges-proskauer	Citrate utilization	Urease test	Oxidase test	Motility	Lactose	Mannitol	Glucose	Sucrose	Slope	Butt	H_2S	Gas	Coagulase test	Catalase test	Mannitol salt agar	Hemolysis
Escherichia coli	GNB	+	+	-	-	-	-	+	+	+	+	+	A	A	-	+	NA	+	NA	NA
Proteus mirabilis	GNB	-	-	-	+	+	-	+	-	-	+	+	K	A	+	+	NA	+	NA	NA
Klebsiella pneumonia	GNB	-	+	+	+	+	-	-	+	+	+	+	A	A	-	+	NA	+	NA	
Pseudomonas aeruginosa	GNB	-	-	-	+	-	+	+	-	+	-	-	K	K	-	-	NA	+	NA	+
Staphylococcus aureus	GPC clusters	-	+	+	N A	N A	N A	-	+	+	+	+	NA	NA	NA	NA	+	+	+	+
Staphylococcus saprophyticus	GPC	N A	N A	N A	N A	N A	N A	-	+	+	-	+	NA	NA	NA	NA	-	+	-	-

Biochemical characteristics of yeast

Isolate	Gram stain	Fermentation					Assimilation						Other reactions					
		Dextrose	Maltose	Sucrose	Lactose	Galactose	Dextrose	Maltose	Sucrose	Galactose	Lactose	Urease	Pseudohyphase	Growth at 37°C	Germ tubes	Indian ink	Capsule
Candida albicans	Gram positive oval shaped cocci	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-	-

KEY + Positive - Negative NA Not Applicable K Alkaline (Red-pink) A Acid (Yellow).

Discussion

Sexually transmitted infections occur worldwide, with more than 340 million new cases which are curable [13]. Sexually transmitted diseases are prevalent in sub-Sahara Africa especially Nigeria. Sexually transmitted infection include gonorrhoeae, syphilis, trichomoniasis, urethritis and Human Immunodefieciency Virus (HIV)[14].

Urethritis is the inflammation of urethra, which usually occurs in both men and women. Urethritis can be infectious and noninfectious. Non-infectious urethritis appears during the damages of urethra with the diagnostic and therapeutic procedures, also as reaction to food and drug allergens. For infectious urethritis which is the focus of this study. Infection of the anterior urinary tract (urethritis) is mainly caused by *Neisseria gonorrhoeae*, Staphylococci, Streptococci and Chlamydiae [8,15]. In this study however, *Neisseria* was not isolated from any clinical specimen investigated. The reason may be due to antibiotic abuse. It is difficult to recover this microorganism in culture after antibiotic intake, moreover, symptomatic treatment of sexually transmitted disease is common in Nigeria[16] and *Nesseria gonorrhoeae* is difficult to recover from surveillance culture, after antibiotic intake. Other microorganism found in urethral discharges include Staphylococci, streptococci, *Escherichia coli* and *Candida albicans*, [17,18]. Eventually, microorganism isolated from this study included *Staphylococcus aureus, S. saprophyticus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Esherichia coli, Proteus mirabilis and Candida albicans.* Specimens collected included urine sample, and urethral swabs.

Escherichia coli (31.6%) isolated as agent of nongonococcal urethritis. Previous studies have established the occurrence of *Escherichia coli* in urethral smear of men with urethritis, Dan *et al.* (2012)[19], reported the isolation of *E. coli* by culture and confirmed by pulse-field gel electrophoresis of urethral discharge and urine of a man with urethritis and orchiepididymitis, with a normal urinary tract. Similarly, *E. coli* was detected in men who were homo and bisexual individuals. About 62% of cases of population studied, presented with urethritis in addition to or preexisting cystitis [20].

S. aureus (19.5) was detected as agent of non gonococcal urethritis .*S. aureus* was detected as causative agent of nongonococcal urethritis, of 202 cases of Gram positive cocci, *S. aureus* was recovered from 178 of cases from men presenting with urethritis in a health centre in Nigeria. Ivanor, (2007)[21] isolated Coryneforms coagulase negative staphylococci, streptococci, and *Lactobcaillus* spp from healthy men aged between 18 and 24. While *Staphylococcus aureus*, enterococci, micrococci and enterobacteriaceae (*Escherichia coli*, Enterobacter spp, *Enterococcus feacalis*) were solated from group of men with non-chlamydial, non-gonococal urethritis or persistent non specific urethritis. *Staphylococcus aureus, Escherichia coli, Enterobacter* spp, *Micrococcus spp, Enterococcus feacelis* were not isolated from the healthy men.

Candida albicans (15.0%) was also isolated from the clinical specimens in this study. *Candida* species especially *Candida albicans* are found in small numbers in the commensal flora (mouth, gastrointestinal tract, vagina, skin) of about 20% of the normal population [22]. *Candida* can be transmitted sexually and so has been listed by the Centre of Disease Control and Prevention as a sexually transmitted disease patogen[23] . *Candida albicans* proliferates faster due to altered condition of the host as such cause disease. Genitourinary tract infection is prevalent in females during adolescence and childbearing years [24]. *Candida albicans* has been mentioned as one of the

causative agents of urethritis[18] . *Candida* species were isolated in 30 patients with nongonococcal urethritis who had not responded to classical antimicrobial therapy[25].

Proteus mirabilis (13.5%) was isolated, as well. *P. mirabilis* inhabits the human urinary tract where it is believed to cause urinary tract infection associated with the formation of renal and bladder calculi. *P. mirabilis* require an alkaline environment[26] . The flagellum of *P. mirabilis* is important to its motility and is able to colonize the surfaces it comes in contact. The flagellum has been linked to the ability of the organism to form biofilms aiding in the bacteria resistance to defenses of the host and selected antibiotic. *P. mirabilis* also uses pili to adhere to the host urinary tract. Ability to possess urease enable the organism to thrive due to raised pH. Increase pH allows stone formation to take place. On occasion, the stones fill the entire renal pelvis. *P. mirabilis* infection occurs when the bacterium moves to the urethra and urinary bladder. Though majority of urinary infection are due to *E. coli*, urinary tract infection caused by *P. mirabilis* occur in patient with long-term catheterization, where it creates encrustation on the urinary catheters and thereafter block the catheter. Symptoms of urethritis are mild and include; frequency of urination and pyuria (presence of white blood cells in the urine)[26]

P. aeruginosa and S. saprophyticus (7.5%) P . aeruginosa is present on the skin of the axila and perineum in some persons. It also infect the ear, joint, vagina and cervix [27]*P. aeruginosa* was identified as agents of non gonococcal urethritis in this study, though not previously mentioned in previous studies. *S. saprophyticus* has also been isolated from men attending sexually transmitted disease clinic. The urethral smear of these men had 10 leukocytes per high power focus[28]). *K. pneumonia* (5.3%) isolated in this research has been implicated as agent of urethritis.

Conclusion

Urethritis as a sexually transmitted disease cause morbidity and mortality in young population. This group of people among the general population are sexually active. Isolation and identification of causative agents of this disease is important in microbial diagnosis. When the disease causing agents are known and treated, morbidity and mortality as a result of urethritis will be reduced. The transmission process will be intercepted and therefore limit the spread of disease in the general population. Non gonococcal agents of urethritis isolated and identified using cultural and biochemical method included the following microorganisms: *Escherichia coli* (31.6%) *Staphylococcus aureus* (19.5%), *Candida albicans* (15.0%), *Proteus mirabilis* (13.5%), *Pseudomonas aeruginosa* (7.5%), *Staphylococcus saprophyticus* (7.5%) and *Klebsiella pneumoniae* (5.3%).*Pseudomonas aeruginosa* have not been mentioned previously as agent of non gonococcal urethritis. Further study on molecular identification of non gonococcal urethritis shall be reported later.

References

1. Terries MK Urethritis *emedicine* (2006) 1-19 http:www.emedicine.com/med/topics2342.htmaccessed 25th July 2012.
2. Thomas E and Herchline MD. Urethritis: Epidemiology. Infectious Disease Antimicrobial Agents.www.antimicrobe.org? e14.asp 2014.
3. McCutchan JA Epidemiology of veneral urethritis comparison of gonorrhoea and non gonorrhoea urethritis. *Review of infectious Diseases* 1984; 6: 669-88.
4. Thompson SE Washington AE Epidemioly of sexually transmitted *Chlamydia trichomatis* infections.

Epidemiogical Reviews 1983; 5: 96-123.

5. Weisner PJ Selected Aspects of the Epidemiology of non gonococcal urethritis in Hobson D, Holmes K.K (eds). *Nongonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology 1977 pp 9-13.
6. Choudry S,Ramachanran VG, Das S Bhattachaya SN and Moghe NS Patterns of sexually transmitted infections and performance of syndromic management against etiological diagnosis on patient attending the sexually transmitted infection clinic of a tertiary care hospital. *Indian Journal of Sexually Transmitted Diseases* 2010;31:104-108.
7. Horner P, Blee B Ch, O Mechinary C, Muir P, Evans C. and Radcliffe K. *European Guideline on the management of non-gonococcal* 2014 *urethritis*http://ww.iusti.org/ regions/europe/word_docs/2014/European_guidelineNGU_draft_10102014.pdf, accessed 20thApril , 2015.
8. Cheesebrough M Examination of Urine In *District laboratory practice in tropical countries* part 2. Cambridge University Press. 2004; pp105-115.
9. Cowan S T, Steel K J, Barrow G I. and Feltham R K A. *Cowan and Steel's manual for the identification of medical bacteria* Cambridge University Press, Cambridge. 1993
10. Kauffman, C.A, Fisher, J.F, Sobel, J.D. and Cheryl, A. Newman. *Candida* Urinary Tract Infections--Diagnosis. *Clin Infect Dis* 2011; 52(6): 452-456.
11. Eddy AD and Mustapha M. Phenotypic identification of *Candida* species and their susceptibility profile in patients with genitourinary candidiasis. *International Journal of Advanced Research* 2014; 2(12) 76-84.
12. Holt, J. G., Krieg, N.R., Sneath, P.H., Safety, J.T., Withians, S.T *Bergey's manual Determinative Bacteriology*. Williams K, Wilkins, O. (Eds), Baltimore. 1993.
13. WHO, Sexually transmitted infections (STIs) fact sheet N0° 110: World Health Organization. Available: www.who.int /.../fs110/, 2103.
14. Nduche M U and Okwulechie I. Ethnomedical Survey of plants used in treating Sexually transimitted diseases in Abia State Nigeria. *Review of Plant studies*. 2014;1(1):1-9
15. Okolie CU and Iroegbu CU Evaluation of extracs of Anthocleistra djalonensis, Nauclea latifolia and Uvaria afzalii for activity against bacterial isolates from cases of nongonococcal urethritis. *Journal of Ethnopharmacology*. 2004; 92 (1):135-44
16. Ajayi AA, Fayemiwo SA, Bakare RA .International Union Against Sexually Transmitted infections Africa (IUSTI-Africa) *Newsletter*, Issue 2002;11-12.
17. Major MPV and Bombey DVD .Non-gonococal urethrities.*The Licentiate* 1963*;* 13(4): 109-111.
18. Varela JA, Otero L. and Garcia MJ Trends in the prevalence of pathogens causing urethritis in Asturias, Spain, 1989–2000 *Sexually Transmission Diseases,* 2003. 30:280–283.
19. Dan M, Gottesmen T, Schwartz D, Tsivian A, Gophna U. and Rokney A. Sexually transmitted *Escherichia coli* urethritis and orchi epidimititis *Sexually transmitted disease,* 2012; 39 (1).
20. Barnes RC, Daifuku R, Roddy RE. and Stamm W E Urinary tract infection in sexually activity in horizontal men *Lancet* 1986; 1*:* 171-173.
21. Ivanov B . Microbiological features of persistent non specific urethritis in men. *Journal of Microbiology Immunology and*

Infection. 2006; 40: 157-161.

22. Greenwood D, Black RC B. and Peutherer, *Medical Microbiology 16th edition.A guide to Microbial Infections, Pathogenesis, Immunity, Laboratory Diagnosis and Control*. Elsevier, Churchill Livingstone.2002
23. Prescott ML, John P, Herlys T, Klein. *Human diseases caused by Bacteria in Microbiology* 7th ed 2008; p 973-976. McGraw-Hill international Edition St Louis, Toronto:
24. Sobel JD, Wiesenteld H C, Martens M, Danna P, Hooton, I M, Rompalo A Sperling M, Livengood C, Horowitz B, Thron JV, Edwards L, Paizer H. and Chu T C. Maintenance fluconzole therapy for recurrent vulvovaginal candidiasis. *The New England Journal of Medicine* 2004; 357:876-883.
25. Beduk Y, Manalp M. Detection of candidiasis in non-gonococcal urethritis resistant to therapy *Mikrobiyoloji Buteni* 1986; 20(3): 190-5.
26. Liaw ST. Inhabitation of virulence factor expression and swarmming expressing in *P. mirabilis* by p- nitrophynol. *Journal of Medical Microbiology*. 2000; 49: 775-731.
27. Ananthanarayan R. and Paniker CKJ *Pseudomonas* in Ananthanarayan R, Paniker C.K.J. Editiors. *Textbook of Microbiology* Hyderabad: University Press India) prirate limited: Eight Edition 2009; 315-319.
28. Hovelius B, Thein I and Mardh PA *Staphylococcus saprophyticus* in the etiology of non gonococcal urethritis. *British Journal of Veneral Diseases*. 1979; 55:369-374.

Citation: this article should be cited as. *Adomi, Patience O*. Isolation and Identification of Non-gonococcal Organisms Associated with Urethritis from Clinical Samples. Afr. J. Trop. Med. & Biomed. Res 2019; 4 (2): 17-25