Nongonococcal And Nonchlamydial Microbial Isolates From High Vaginal Swabs Of Nigerian Women Diagnosed With Pelvic Inflammatory Disease

C Uneke, M Alo

Citation

C Uneke, M Alo. *Nongonococcal And Nonchlamydial Microbial Isolates From High Vaginal Swabs Of Nigerian Women Diagnosed With Pelvic Inflammatory Disease*. The Internet Journal of Infectious Diseases. 2006 Volume 6 Number 1.

Abstract

High vaginal swabs from 1451 Nigerian women clinically diagnosed with pelvic inflammatory disease (PID) were investigated for nongonococcal and nonchlamydial microbial infections using standard techniques. Bacteria were isolated from 623(42.9%, 95% CI., 40.4-45.4%) women, comprising 474(76.1%) monobacterial and 149(23.9%) polybacterial isolates. Trichomonas vaginalis and Candida albicans were isolated from 124(8.5%, 95% CI., 7.1-9.9%) and 611(42.1%, 95% CI., 39.6-44.6%) women respectively. Predominant bacterial isolates were Escherichia coli (34.9%, 95% CI., 29.2-40.6%) and Staphylococcus aureus (27.1%, 95% CI., 24.0-30.2%), while least bacterial isolates were Streptococcus species (5.1%, 95% CI., 3.5-6.7%) and Gardnerella vaginalis (4.3%, 95% CI., 2.9-5.7%). Individuals aged 36-40 years were significantly more infected with bacteria (χ^2 =107.97, P<0.05) and C. albicans (χ^2 =55.90, P<0.05). While prevalence of T. vaginalis was significantly higher among individuals aged 26-30 years (χ^2 =27.46, P<0.05). Routine screening and treatment of women for lower genital tract infections to minimize their role in PID is recommended

INTRODUCTION

Pelvic inflammatory disease (PID) is the most important complication of the female genital tract, causing major medical, social and economic problems worldwide [1]. PID comprises a spectrum of inflammatory disorders of the upper female genital tract, including any combination of endometritis, salpingitis, tubo-ovarian abscess, and pelvic peritonitis [2,3]. PID is a polymicrobial infection due to the ascending of normal endogenous microorganisms from the lower genital tract into the upper genital tract or the infection by microorganisms related to sexually transmitted diseases (STD) as Chlamydia trachomatis and Neisseria gonorrhoeae [4,5]. Complications of PID are common and difficult to treat and include tubo-ovarian abscess, ectopic pregnancy, recurrent PID and infertility [5]. Overall, such complications are estimated to occur among 15%-20% of women with PID, and are associated with great emotional stress and can have a major effect on a woman's reproductive health [6]. Approximately 12% of women are infertile after a single episode of PID, almost 25% after two episodes, and over 50% after three or more episodes [7].

Despite advances in defining its aetiology, pathogenesis and availability of many powerful antimicrobial drugs, PID consumes a significant portion of the medical resources of numerous countries [8]. In US for instance, at least 5.5 billion dollars are spent on PID annually and more than a million women are diagnosed with PID each year and for every four women who have PID, one will suffer a complication [1,9].Often the PID rates are highest in developing countries where medical resources are most severely limited and the number of women with unrecognized PID is estimated to be far higher [8,10]. It is estimated that in developing countries PID is related to 94% of all sexually transmitted infections (STI) related morbidity [11].

There is paucity of information on PID in sub-Saharan Africa and available statistics in the sub-region are rather focal [12,13]. This is largely attributed to the fact that clinical diagnosis of PID is at best difficult and imprecise, and laboratory criteria are neither highly specific nor sensitive [2,3]. Although it is well established that gonococcal (Neisseria gonorrhoeae) and chlamydial (Chlamydia trachomatis) microorganisms are the major pathogens causing acute PID, currently, there is a rising incidence of nongonococcal and nonchlamydial PID worldwide [13,14,15]. The nongonococcal and nonchlamydial microorganisms

have been reported to be responsible for higher frequency of PID in some areas[14,16]. However, the natural genital flora of females is so varied that determining actual causative agents is difficult. The objective of this study therefore was to investigate the spectrum of nongonococcal and nonchlamydial genital microorganisms implicated in cases suggestive of PID among women most of who are of reproductive age. The public health significance of findings is discussed in the context of reproductive health delivery as it relates to Nigeria and other developing countries of similar setting.

METHODS

Study Population/ Sampling Technique-The study was a two-year hospital-based cross-sectional investigation conducted from January 2003 to December 2004 at Abakaliki the capital city of Ebonyi State, South-eastern Nigeria. The Federal Medical Centre (FMC), one of the largest health institutions and a major referral centre for gynaecological screening, was used for the study. Women who visited the gynaecological unit of the FMC, Abakaliki and were clinically diagnosed with PID, presenting with at least three of the following; lower abdominal tenderness, bilateral adnexal tenderness, uterine tenderness, and cervical motion tenderness [17], were considered for the study. (No laparoscopy or endometrial biopsy was available at the FMC, Abakaliki as at the time of this study). Enrolled women were referred to the diagnostic laboratory unit of the Centre for sampling. High vaginal swab (HVS) was obtained from each woman. Each patient was given a sterile cotton-tipped swab and instructed to insert the swab into the vagina and to swab the vaginal wall, as described in an earlier study [18]. The age of each subject was obtained by interview.

Approval for the study was obtained from the Research/Ethical Committees of the FMC, Abakaliki. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice/quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. Prior to sampling, informed consent was duly obtained from each subject.

Laboratory Analysis-The swabs were cultured on Blood agar, Chocolate agar and MacConkey agar media according to standard protocol described previously [19]. The Blood agar and MacConkey agar media were incubated aerobically at 37oC for 24 hours, while the Chocolate agar media were incubated in a carbon dioxide atmosphere. A canister with candle was used to create the carbon dioxide. A wet preparation was also made from each swab by placing it in 1.5 ml of sterile phosphate-buffered saline (PBS), pH 7.2. The resulting suspension was used to produce a wet mount for direct microscopic examination. Colonial characteristics, Gram reaction, haemolysis on Blood agar media, and biochemical tests such as catalase test, coagulase test, indole tests, citrate utilization, urase activity, and oxidase test were conducted as described previously [19] for microbial isolation and identification.

Statistical Analysis- Differences between proportions were tested using Chi-square. Statistical significant was achieved if $P < 0.05$.

RESULTS

A total of 1451 women diagnosed with PID were studied. The age ranged from 20-54years old. Bacterial organisms were isolated from 623(42.9%, 95% CI., 40.4-45.4%) HVS specimens of the patients, comprising of 474(76.1%) monobacterial and 149(23.9%) polybacterial isolates. Trichomonas vaginalis and Candida albicans were isolated from 124(8.5%, 95% CI., 7.1-9.9%) and 611(42.1%, 95% CI., 39.6-44.6%) HVS specimens of the patients respectively. A total of 768 bacterial isolates were made consisting of both gram-positive and gram-negative bacteria. The predominant bacterial isolates were Escherichia coli (34.9%, 95% CI., 29.2-40.6%) and Staphylococcus aureus (27.1%, 95% CI., 24.0-30.2%), while the least bacterial isolates were Streptococcus species (5.1%, 95% CI., 3.5-6.7%) and Gardnerella vaginalis (4.3%, 95% CI., 2.9-5.7%) (Table 1).

When age of the women diagnosed with PID was associated with the different microbial isolates, higher prevalence of bacteria isolates was noted among the older women, with the highest prevalence in the 36-40 years age category (68.7%, 95% CI., 63.3-74.1%) and least prevalence among those aged 26-30 years old (28.8%, 95% CI., 22.5-35.1%) (Table 2). A statistically significant difference was also observed in the trend ($?2 = 107.97$, df=6, $P<0.05$). The prevalence of T. vaginalis was highest among individuals of the 26-30 years age group (13.6%, 95% CI., 8.8-18.4%), followed by the 31-35 years age category (10.3%, 95% CI., 7.0-13.6%) (Table 2), and there was a statistically significant difference in the trend ($?2 = 27.46$, df=6, $P<0.05$). The prevalence of C. albicans was highest among women of the 36-40 years age group (57.4%, 95% CI., 53.5-61.3%) and least among those aged 20-25 years old (28.1%, 95% CI., 24.5-31.7%) (Table

2), a statistically significant difference was also observed in the trend (?2 =55.90,df=6,P<0.05).

Figure 1

Table 1: Spectrum of nongonococcal and nonchlamydial bacterial isolates from high vaginal swab specimen of women with PID in Abakaliki, Nigeria.

Escherichia coli	268	34.9	29.2-40.6
Staphylococcus aureus	208	27.1	24.0-30.2
Streptococcus species	39	5.1	3.5-6.7
Proteus species	107	13.9	11.5-16.3
Pseudomonas species	71	9.2	7.2-11.2
Klebsiella species	42	5.5	3.9-7.1
Gardnerella vaginalis	33	4.3	2.9-5.7
Total	768	52.9	50.3-55.5

Figure 2

Table 2: Age-related prevalence of microbial isolates from high vaginal swab specimens of women with PID in Abakaliki, Nigeria.

Age of Women (yrs)	Number examined	Number (%) with Bacterial infection	95% Confidence interval	Number (%) with *T. vaginalis*	95% Confidence interval	Number (%) with *C. albicans*	95% Confidence interval
20-25	135	41(30.4)	22.6-38.2	12 (8.9)	7.9-25.7	38 (28.1)	24.5-31.7
26-30	198	57 (28.8)	22.5-35.1	27 (13.6)	8.8-18.4	92 (46.5)	42.5-50.5
31-35	310	112 (36.1)	30.8-41.4	32 (10.3)	7.0-13.6	122 (39.4)	35.5-43.3
36-40	284	195 (68.7)	63.3-74.1	28 (9.9)	6.4-13.3	163 (57.4)	53.5-61.3
41-45	184	76 (41.3)	34.2-48.4	16 (8.7)	4.6-12.8	82 (44.6)	40.7-48.5
46-50	173	73 (42.2)	34.9-49.5	9 (5.2)	1.9-8.5	63 (36.4)	32.6-40.2
>51	167	69 (41.3)	33.8-48.8	0 (0.0)	-	51 (30.5)	26.8-34.2
Total	1451	623 (42.9)	40.4-45.4	124 (8.5)	7.1-9.9	611 (42.1)	39.6-44.6

DISCUSSION

The aetiology of PID has been described as multimicrobial, and the pattern of organisms most frequently causing the condition fluctuates [16]. This is evident from this study where bacterial, parasitic and fungal agents were isolated from the HVS and may be implicated in the PID diagnosed in the patients. This has a very important public health implication because clinicians often face the problem of knowing the exact aetiology of PID in order to treat appropriately. It has, therefore been suggested that in addition to the major PID causative agents (N. gonorrhoeae and C. trachomatis), a proper isolation and identification of other potential microbial pathogens achieved by culture of lower genital tract samples could lead to a better, specific and proper treatment of this disease [4]. Furthermore, it is our submission that chlamydial and gonococcal interventions alone are unlikely to eradicate PID.

In this study, microbial isolates of bacterial and fungal origin predominated with higher percentage of women with bacteria (42.9%) and C. albicans (42.1%) compared to percentage with T. vaginalis (8.5%). This was not surprising, because the female genital tract has diverse microenvironments propitious for growth of different types of aerobic and anaerobic bacteria as well as C. albicans (Candida vaginitis) and in PID patients, each anatomic site develops specific features conditioning their growth [13,20]. Although T. vaginalis occurrence was comparatively low in this investigation, it has been implicated in atypical PID in infertile women [21]. Furthermore, because bacteria have been demonstrated to be attached to T. vaginalis in vitro [22], it has been postulated that trichomonal infection may facilitate the ascent of C. trachomatis and increased risk of developing clinical PID among women infected with C. trachomatis [23]. In addition, reports have indicated that most of the microorganismms isolated in this study are not only frequent secondary invaders following an initially sexually transmitted infection, but can also primarily cause PID [24,25]. As a public health measure therefore, a policy of routinely screening women for lower genital tract infections should be pursued as part of secondary prevention strategy to keep lower genital tract infection from moving up to the upper genital tract where they can cause PID.

E. coli and Staphylococcus aureus were the most predominant bacteria isolated from the PID patients in this study, and this was in conformity with findings from similar studies in Gombe, Nigeria [13], Haryana, India [8] and Ioannina, Greece [15]. Interestingly, however, polybacterial cultures of up to 23.9% were obtained. This is comparable to findings from three similar studies in various parts of India where polybacterial cultures were obtained in 21.8% [26], 42.0% [27] and 43.2% [8] of the PID cases investigated. The implication of the occurrence of polybacterial cultures in PID cases is that bacterial synergism and antibiotic resistance make the selection of an optimal antibiotic regimen difficult [28], especially in the developing countries including Nigeria, where inadequate health services, inadequate drug supplies, non-adherence to treatment strategies and dubious drug quality all favour the emergence of microbial resistance [29]. Although age is inversely related to PID rates and directly correlated with PID sequelae, (e.g., tubal damage and infertility) [30], our result indicated that older women appeared more likely to have significantly higher prevalence of bacterial infection (P<0.05). The prevalence rates of C. albicans and T. vaginalis may also be described as fairly high among the infected older women and differences were also significant (P<0.05). Swinker [31] had earlier noted that epidemiologic characteristics of the various microorganisms implicated in PID differ, with the frequency

of nongonococcal disease higher in older women. Hence it is our opinion that in older women (assumed to be less sexually active), the nongonococcal and nonchlamydial microorganisms found in the lower genital tract may be responsible for a considerable number of the PID cases. More operational research using both molecular and immunological tools to fully elucidate this is advocate.

In conclusion, it is worth noting that a major limitation in this study was our inability to adequately prove that the microorganisms isolated were responsible for the PID diagnosed. A more complex study to achieve this goal using molecular biologic tools is advocated. Secondly, the non inclusion of women without PID in this study which would have provided a basis for comparison was yet another drawback. This is advocated in future studies. Thirdly, the Hager et al. [17] definition of PID used in this study, lacks specificity and consequently some patients included in this study may not have had PID. This problem is reportedly inherent to all studies of PID [32]. Future studies that would investigate the pathological basis of the relation between the nongonococcal and nonchlamydial microorganisms of the lower genital tract and PID using samples from women with PID diagnosed using laparoscopy and endometrial biopsy are advocated. Since PID is a complex syndrome that encompasses a broad spectrum of inflammatory diseases (e.g., endometritis, salpingitis, and tubo-ovarian abscess) that may be caused by a variety of organisms, guidelines for the treatment of patients with PID, therefore, need to be designed to provide flexibility in therapeutic choices. In addition to providing broad-spectrum coverage of likely etiologic pathogens, selection criteria for a treatment regimen should also include institutional availability, cost-control efforts, patient acceptance, and regional differences in antimicrobial susceptibility [6].The prevention of PID is a direct result of the prevention and prompt recognition and treatment of STDs or of any suspected infection involving the female genital tract. Treatment for sex partners of women with PID is also imperative for failure to manage their sex partners effectively places the women at risk for recurring infection and related complications. Lifestyle changes should be geared to preventing the transfer of organisms when the body's delicate lining cells are unprotected or compromised and the use of barrier contraceptives, such as condoms is encouraged.

ACKNOWLEDGEMENT

We wish to thank the authorities of the Federal Medical Center Abakaliki for logistical support.

CORRESPONDENCE TO

CJ Uneke Department of Medical Microbiology/Parasitology, Faculty of Clinical Medicine, Ebonyi State University, PMB 053 Abakaliki, Nigeria. Tel: 234-08038928597, Fax: 234-04300222, E-mail: unekecj@yahoo.com

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Author Information

Chigozie J. Uneke, M.Sc.
Department of Medical Microbiology/Parasitology, Faculty of Clinical Medicine, Ebonyi State University

Moses N. Alo, M.Sc.
Federal Medical Centre