MICROBIAL SPECTRUM OF PELVIC INFLAMMATORY DISEASES IN NGURU, NIGERIA

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ABSTRACT
Pelvic inflammatory diseases, a leading gynecological problem worldwide, are associated with socio-economic and psychological costs. A retrospective study of 1350 high vaginal swabs analyzed between Jan-Dec. 2005, showed that 845 (62.8%) were positive for 9 microorganisms by culture/or wet preparation. Microbial growth was found in 645 (76.3%) cases. Polymicrobial growth was found in 90 (10.7%) cases, and 3(0.4%) yielded anaerobic growth. Staphylococcus aureus accounted for 355 (42.0%) cases, followed by Escherichia coli 190 (22.5%), Trichomonas vaginalis 100 (11.8%) Candida spp and Neisseria gonorrhoeae 70 (8.3) and the least, Pseudomonas spp 5 (0.6%) Microbial-associated infection was prominent in the group 21-30 years old (46.6%) and 31-40 (23.9%) years respectively. Antibiotic susceptibility pattern showed that mean susceptibility greater than 50% were recorded with ofloxacin 80%, ceftazidime 80%, rifampicin 81.9% compared to mean susceptibility less than 50% recorded with trimethoprim-sulthamethoxazole 34.7%, and ampicillin 26.1%.

In conclusion, the reported microbial-associated infection in PID with a prevalence of 62.8% is of public health importance. Early diagnosis of causative agents and prompt institution of chemotherapeutic agents will help to prevent clinical complications that are expensive to treat.

Keywords: pelvic inflammatory diseases, microorganisms, antibiotic susceptibility.

INTRODUCTION
Pelvic inflammatory disease (PID), is an infection of the upper genital tract in women that include endometritis, parametritis, salpingitis, oophoritis, tubo-ovarian abscess and peritonitis (1,2). It accounts for 5-20% of hospital admissions for gynecological problems in general/gynecological clinics worldwide (3). In USA, infertility that affects approximately 10-15% of all couples attribute tubal damage due to pelvic infection (4,5). Clinical presentation varies in severity, and ranges from sub clinical, asymptomatic infections exerting medical and psychological cost that include chronic pelvic pain, ectopic pregnancy and infertility (1). It has been associated with increase risk of ovarian cancer6-8. The pathogenesis is complex interaction of genetic, immunological and bacterial virulence factors (9).
The prevalence and incidence of PID varies greatly, because of significant misdiagnosed/or unreported cases. In developed countries, annual incidence of PID increased in women aged 15-45 years, with peak of infection in 20-24 years (10). Polymicrobial agents are associated and initiated pathogenesis of PID, particularly in presence of facultative aerobic and anaerobic bacterial isolates (11-14), with *Niesseria. Gonorrhoea* and *Chlamudia tracomatis* as leading pathogens, accounted for 60-80% in women of aged less than 25 years12-14. Other less pathogenic mycoplasma, and endogenous aerobic and anaerobic bacteria have also been implicated (15). Co-existence of sexually transmitted diseases (STD) etiological agent in genital tract predispose the women to acquisition of PID (1,16). Korn *et al* (17) reported that clinical presentation and course of PID in women with symptomatic HIV disease and/or severe immune suppression may be more aggressive than in HIV negative women.

Clinical diagnosis is rather difficult, as no single clinical and laboratory test in definite as gold standard, thus combination of test seems to improve sensitivity and specificity (18,19). Epidemiological and microbiological indices associated with PID are important source of preventable reproductive infertility in women, and other clinical squealed. Little information is available on PID epidemiology in this environment, this there is no baseline in assessment of its relationship in case of infertility and HIV infection.

Early diagnosis/treatment of PID could stemmed down the effect on the fallopian tubes; and in case of microbe-related inflammation and tubal necrosis can similarly precedes manifestation of symptoms, especially in aetiological agent due to chlamydial3. Prompt diagnosis and institution of appropriate antibiotic therapy would prevent possible sequelae of PID. The retrospective study examined the aetiological spectrum in high vaginal swabs of pelvic inflammatory diseases in this environment.

**MATERIALS AND METHODS**

**Study Site**

The retrospective study was conducted in Federal Medical Center, Nguru, between Jan-Dec 2005, which involved the Pathology and Obstetric/Gynecology departments. The patients folder presented at the general out-patients/gynecology clinic, with clinical complaint suggestive of pelvic inflammatory diseases, ranged from pelvic vaginal discharge to lower abdominal pain, with high vaginal swabs collected and sent for bacteriological analysis. Criteria of inclusion are consecutive non-duplicate high vaginal swabs, repeated swab analysis and mixed growth of doubtful significance were excluded. Information retrieved from the patients folder included age, sex, and clinical complaint.

**Processing of the Specimens**

The high vaginal swab was processed, with inoculation on Blood, Chocolate and Sabouraud agar plates, incubated at 37°C for 24hours. Bacterial/yeast were identified by standard bacteriological and mycological techniques (20-22). Yeats were further identified by germ tube
test. Direct smear was prepared stained by Gram methods, and wet preparation of the specimen for parasitic examination. Antibiotic susceptibility testing was determined by disc diffusion, using the following antibiotic discs, ofloxacin (OFX), ciprofloxacin (CPX), pefloxacin (PEF), ceftazidime (CAZ), cefuroxime (CXM), rifampicin (RF), streptomycin (S), tetracycline (TET), trimethoprim sulthamethoxazole (SXT), ampicillin (AMP), gentamycin (CN), erythromycin (E), and augmentine (AU). The zone of inhibition of the disc was measured to determine whether resistant or sensitive in accordance to NCCLS guidelines (23). The mean susceptibility percentage of each antibiotic was calculated as the number of bacterial isolates susceptible divided by total number of bacterial isolates tested multiply by 100.

**Data Analysis**

Data and information retrieved from patients folders were entered into study database using SPSS version 13.0. The value were expressed mean and percentage, and appropriate statistical package where necessary.

**RESULTS**

Of the 1350 high vaginal swabs results analyzed, 846 (62.8%) were positive for 9 microorganisms by culture/or wet preparation examination (7 bacterial pathogens, 1 fungi and 1 protozoan) as in table 1. The means age of the patient was 22.4 + 2.7 years. The ratio of gram-negative bacteria ratio was 1:2.5 Monomicrobial growth was recorded in 645 (76.3%), polymicrobial growth in 90 (10.7%) and bactero-fungal in 110 (12.0%) cases. Three (0.4%) cases yielded anaerobic growth.

*Staphylococcus aureus* was the accounted for 355 (41.9%) cases, followed by *Escherichia coli* 190 (22.4%), *Trichomonas vaginalis* 100 (11.8%), *Neisseria gonorrhoeae* and *Candida spp* 70 (8.3%) repetitively. Microbial-associate infection distribution, in accordance with the age group of the patients studied (table II), frequency of occurrence was predominant with the age group 21-30 (46.7%) and 31-40 (23.9%) years and least in 10-20 (8.1%) and >51 (6.4%) years respectively. There was statistically significant difference between microbial infection and the age-group (p<0.05). Similarly, there was a decreasing trend pattern in frequency of occurrence of microorganism and age-group.

Antibiotic susceptibility pattern of bacterial isolates as shown in table III, showed that mean susceptibility percentage greater than 50 was observed with ofloxacin, gentamycin, ciprofloxacin, pefloxacin, rifampicin, cefuroxime, ceftazidime, erythromycin and streptomycin, and less than 50 in trimethoprim-sulthamethoxazole, tetracycline, ampicillin, and augmentine.
Table I: Frequency of occurrence of Microorganisms Isolated

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Frequency of Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive bacteria (n=370)</strong></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>355 (42.0)</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>15 (1.8)</td>
</tr>
<tr>
<td><strong>Gram-negative bacteria (n=305)</strong></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>190 (22.5)</td>
</tr>
<tr>
<td>Neisseria gonorrhoea</td>
<td>70 (8.3)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>30 (3.6)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>10 (1.2)</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>5 (0.6)</td>
</tr>
<tr>
<td>Anaerobic bacteria</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td><strong>Fungi (n=70)</strong></td>
<td></td>
</tr>
<tr>
<td>Candida spp.</td>
<td>70 (8.3)</td>
</tr>
<tr>
<td><strong>Parasites (n=100)</strong></td>
<td></td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>100 (11.8)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>848</td>
</tr>
</tbody>
</table>

Table II: Distribution of bacterial isolates according to age-group of patients studied

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>30</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>1</td>
<td>5</td>
<td>16</td>
<td>7</td>
<td>-</td>
<td>69</td>
</tr>
<tr>
<td>21-30</td>
<td>115</td>
<td>10</td>
<td>15</td>
<td>95</td>
<td>8</td>
<td>-</td>
<td>40</td>
<td>74</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>31-40</td>
<td>100</td>
<td>3</td>
<td>8</td>
<td>45</td>
<td>2</td>
<td>-</td>
<td>15</td>
<td>8</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>41-50</td>
<td>75</td>
<td>2</td>
<td>5</td>
<td>25</td>
<td>-</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>&gt;51</td>
<td>35</td>
<td>-</td>
<td>2</td>
<td>15</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>355</td>
<td>15</td>
<td>30</td>
<td>190</td>
<td>10</td>
<td>5</td>
<td>70</td>
<td>100</td>
<td>70</td>
<td>3</td>
</tr>
</tbody>
</table>

Table III: Antibiotic susceptibility pattern of the bacterial isolates (% susceptibility)

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>OF</th>
<th>CN</th>
<th>CI</th>
<th>PE</th>
<th>SX</th>
<th>AM</th>
<th>RD</th>
<th>E</th>
<th>AU</th>
<th>S</th>
<th>CX</th>
<th>CA</th>
<th>TE</th>
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</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>90</td>
<td>65</td>
<td>80</td>
<td>89</td>
<td>25</td>
<td>21</td>
<td>89</td>
<td>75</td>
<td>60</td>
<td>70</td>
<td>85</td>
<td>72</td>
<td>45</td>
</tr>
<tr>
<td>E. coli</td>
<td>82</td>
<td>70</td>
<td>75</td>
<td>83</td>
<td>43</td>
<td>26</td>
<td>78</td>
<td>65</td>
<td>72</td>
<td>73</td>
<td>78</td>
<td>80</td>
<td>54</td>
</tr>
<tr>
<td>Kleb spp</td>
<td>78</td>
<td>56</td>
<td>78</td>
<td>78</td>
<td>45</td>
<td>35</td>
<td>85</td>
<td>67</td>
<td>56</td>
<td>67</td>
<td>76</td>
<td>82</td>
<td>42</td>
</tr>
<tr>
<td>Strep spp</td>
<td>90</td>
<td>82</td>
<td>89</td>
<td>79</td>
<td>50</td>
<td>42</td>
<td>90</td>
<td>90</td>
<td>80</td>
<td>80</td>
<td>84</td>
<td>79</td>
<td>35</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>75</td>
<td>67</td>
<td>75</td>
<td>74</td>
<td>35</td>
<td>22</td>
<td>76</td>
<td>69</td>
<td>67</td>
<td>67</td>
<td>73</td>
<td>80</td>
<td>45</td>
</tr>
<tr>
<td>Pseudo spp</td>
<td>60</td>
<td>42</td>
<td>73</td>
<td>67</td>
<td>20</td>
<td>12</td>
<td>65</td>
<td>52</td>
<td>45</td>
<td>45</td>
<td>75</td>
<td>82</td>
<td>38</td>
</tr>
<tr>
<td>N.gonorrhoeae</td>
<td>85</td>
<td>65</td>
<td>73</td>
<td>74</td>
<td>25</td>
<td>25</td>
<td>90</td>
<td>68</td>
<td>50</td>
<td>78</td>
<td>80</td>
<td>85</td>
<td>38</td>
</tr>
<tr>
<td><strong>Mean Susceptibility (%)</strong></td>
<td>80</td>
<td>63</td>
<td>77</td>
<td>77</td>
<td>34</td>
<td>26.1</td>
<td>81</td>
<td>69</td>
<td>69</td>
<td>68.6</td>
<td>78.7</td>
<td>80</td>
<td>42.2</td>
</tr>
</tbody>
</table>

DISCUSSION

Clinical significance of PID becomes pronounced because its association with sexually transmitted diseases/HIV/AIDS infections. Particularly in asymptomatic individuals who may later present with various complications irrespective of the social and psychological cost (19,24). Consequently, a dramatic increase in the
the incidence of PID has led to a parallel increase in infertility (25).

The reported prevalence of microbial-associated infection in PID of 62.8% of our patients is high. Our report is similar to the rates reported in similar studies conducted in Sokoto (26) and Gombe (27) of same geographical zone. However, comparison of PID prevalence in studies conducted at different geographical location/countries might be rather difficult, because of certain inherent biases involved, particularly presence of PID-related infections (10,28-30). Similarly, PID prevalence are influenced by variation in case definition (particularly between different clinical settings), changes in disease chronicity associated with clinically mild Chlamydia infection, variation in health seeking behaviour and increase management of PID in outpatient setting (31,32).

The frequency of occurrence of microbial-associated infection was high in the age group of 21-30(46.7%) and 31-40(23.9%) years. This finding simply confirms reported findings that highest PID prevalence and highest rate of increase are associated and seen in the 16-24 years age groups, and substantial numbers of bacterial sexually transmitted infection are high these age group (16-19,3,6,19,33). Also PID accounts for approximately 60% of gynecological problems in women aged less than 25 years34. High prevalence of PID episodes in sexually active age group, re-emphasizes the correlation that co-existence of aetiological agent in the genital tract of the females predisposes to acquisition of PID (3,19,33-35). Some studies found demographic risk factors associated with PID, like sexual activity at young age, racial, and both pre-delivery history and post-partum diagnosis of chlamydial and gonococci infections (36,37). However implication of these factors in this environment need further evaluation.

From the present study, 10 microorganism (8 bacterial pathogens, 1 fungal and 1 protozoan) were recorded. S. aureus was the commonest and accounted for 42%, polymicrobial infection wasfound in 10.7% of cases and fungal infection in 13.0% of cases. This pattern simply confirms polymicrobial spectrum aetiology associated with pathogenesis of PID (11-14). S. aureus (42.0%), and E.coli (22.5%) were predominant bacterial isolates in the study, these pathogens are most isolated in lower genital tract infections; and are responsible for a significant proportion of sexually transmitted diseases in Nigeria (26,38-40). The dominance of these bacterial pathogens as STI pathogens and their existence in the female genital tract clearly reaffirmed it as a predisposing factor in acquisition of PID (1,16). Polymicrobial infection with other organisms such as anaerobes or facultative aerobes may be initiated by gonorrhea, chlamydia or both (4,5,15,35). The low frequency of occurrence of N.gonorrhoeae as evident in this study, might probably be due to variation in the studied population, method of microbial investigation, variation in severity of the diseases, sampling technology and site of sampling (40). Technically, N. gonorrhoeae is highly fastidious fragile organisms, isolation is dependent on viability of the organism in the specimen, prompt delivery to specimen, and suitability of isolation medium.
Trichomonas vaginalis with a prevalence of 11.6% in a center posed public health problem, because of close association of trichonomasis with HIV infection (42-45). T. vaginalis, is an irritating protozoan and is a common parasitic sexually transmitted disease reported worldwide (45). It is associated with inflammation of the cervix that may mimic cervical tenderness associated with PID (42). Buve et al (43) reported that trichomonasis incidence is higher in cities where there are higher number of HIV-positive individuals. The high prevalence of trichomonasis and candidiasis observed in this study basically revealed close association of poor personal hygienic conditions especially among the low socio-economic class and transmitted sexually, particularly in cases of multiple sex partners (10), with high probability of PID infection.

The in-vitro antimicrobial susceptibility pattern of bacterial isolates revealed that mean percentage susceptibility of greater than 50% was observed with the fluoroquinolones (ofloxacin, ciprofloxacin, perflaxacin), cephalosporins (ceftizidime, cefuroxime) and rifampicin; and those less than 50% observed with gentamycin, erythromycin, augmentine and streptomycin, and least susceptibility observed in tetracycline (42.2%) trimethoprim-sulphahemethoxazole (34.7%) and ampicillin (2.1%). These antibiotic susceptibility patterns are similar to reports by other workers (26,27). The reduced susceptibility of antibiotics like ampicillin, tetracyclines anmd trimthoprim-sulphamethoxazole, clearly revealed the abuse of these agents by self-medication practice, a common norm in most towns/cities in many developing countries.

The fluoroquinolones showed favourable in-vitro susceptibility pattern that could serve as drugs of choice in PID treatment/management. However, documented studies have reported emergence of fluoroquinolones-resistant N. gonorrhoeae (3). With considerable numbers of antibiotics resistant strains, particularly of N. gonorrhoeae to penicillin and cephalosporins (particularly 1st generation), the used of second-generation cephalosporins that posses extended spectrum activity over wide ranged of microorganisms associated with PID3. from the in-vitro antibiotic susceptibility pattern of the study, gentamycin, erythromycin and streptomycin, could served the antibiotic of choice considering the relative cost and also posses extended-spectrum activity. One of the greater threat to the efficacy of antibiotics is the presence/or emergence of resistant strain, thus, cautious approach is required in prescription/administration, as safeguard policy against possible emergence of mutiresistant strain in a remote city, like Nguru.

In conclusion, the prevalence of microbes-associated PID of 62.8%, is high. It is important that microorganisms associated with PID are diagnosed early and appropriate chemotherapeutic treatment/management commenced, as clinical complications are always very expensive to treat.

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