Evaluation of Antimicrobial Agents on Microbial Pathogens of Reproductive Health Importance in a Developing Country

Adenike Adedayo Ogunshe, Ph.D.; Rasheed Bakare, MBBS, FWACP;

1 Applied Microbiology and Infectious Diseases Unit, Department of Botany and Microbiology, University of Ibadan, Nigeria
2 Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria

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Introduction
According to the Center for Disease Control, there are over 15 million cases of sexually transmitted diseases reported annually (1), but in spite of the fact that treatment and control of sexually transmitted infections (STIs) have been improved worldwide with the use of chemotherapeutic agents and immunoprophylactic drugs, the public health implications of infections caused by pathogens of reproductive health importance have not been adequately recognized in Nigeria. Most of such diseases were treatable, but even the once easily cured pathogens that cause reproductive health
infections (RHI) are becoming resistant to many of the older generation antibiotics.

There are presently no regulations guarding the dispensing of clinical drugs in Nigeria, therefore, these particular antibiotics are the most abused because they are easily available and affordable. In addition to the hazards of the resistance among these groups of antibiotics, patients can serve as carriers for long periods of time, thereby transmitting the sexual infections, even across countries and continents. It is also of great concern that most of the traditional herbal practitioners are taking advantage of these situations without basic laboratory findings of their herbal medications (2). The present study was carried out to determine the most potent available antimicrobials in the treatment of pathogens of reproductive health importance in Nigeria.

Materials and Methods

Bacterial isolates

The bacterial flora from urethral (US), eye (ES) and wound swabs (WS) of male patients 2 days to 71 years old as well as cervical swabs (CS/CX) and high vaginal swabs (HVS) of female patients 2 to 86 years old, presenting at the Special Treatment Clinic (STC) of the Department of Medical Microbiology and Parasitology, University College Hospital (UCH), Ibadan, Nigeria were used in this study. Identification of the bacterial isolates was according to standard phenotypic bacterial taxonomic tools (3, 4).

Antibiotic susceptibility determination of bacterial strains

Agar disc diffusion-method: The bacterial isolates from the above-mentioned clinical specimens were screened against the most commonly used, routine antibiotics (discs) in Nigeria [- ampicillin (AMP 10µg); azthromycin (AZM 10µg); ceftriazone (RC 25µg); chloramphenicol (CHL 30µg); ciprofloxacin (CIP 5µg); erythromycin (ERY 5µg); fortum (CAZ 25µg); gentamicin (GEN 10µg); ofloxacin (OFX 25µg); penicillin (PEN 15µg); perfloxacin (PEF 10µg); rocephin (CRO 30µg); septrin (SXT 25mg); streptomycin (S 10µg); tarivid (OFX 30µg); tetracycline (T 30µg); zinnat (CXM 30µg)] according to the Kirby-Bauer and NCCLS methods (5,6). The antifungal susceptibility/resistant patterns of the Candida species [Candida albicans (26.5%); Candida glabrata (18.4%); Candida tropicalis (40.8%) and Candida pseudotropicalis (14.3%)] were phenotypically assayed against 9 commonly available antifungals in the Nigerian markets using the agar well-diffusion methods. The antifungals were [canesten tablet (CNSTT); canesten cream (CNSTC); flagyl (FLGY); mycostatine (MYCS); interzol (INTZ); flucamed (FLMD); mycoten tablet (MYCT); mycoten cream (MYCC) and tetradox (TRDX) tablet.

Results

All the Gram-negative bacterial species associated with reproductive health infections in this study were highly susceptible to ceftriazone (93.3 - 100%); ciprofloxacin (90.0 - 100%); rocephin (87.5 - 100%); perfloxacin (82.4 - 100%); ofloxacin (81.8 - 100%); zinnat (81.3 - 100%); fortum (64.3 - 76.5%) and azthromycin (50.0 - 100%) respectively. Moderate to high susceptibility were exhibited towards chloramphenicol (42.9 - 70.6%) and erythromycin (50.0 - 66.7%), with the exception of Proteus spp. (8.3%) and E. coli (18.2%) which had very low susceptibility towards chloramphenicol; H. ducreyi (25.0%), G. vaginalis (36.2%) and Proteus spp. (30.0%) also had lower susceptibility towards gentamicin. Proteus spp. (8.6%) and G. vaginalis (33.3%) similarly had lower susceptibility towards erythromycin. The most generally resisted of the test antibiotics among the Gram-negative bacteria, based on their phenotypic resistance profiles were gentamicin (40.0 - 75.0%); tetracycline (57.1 - 100%); ampicillin (75.5 - 100%); streptomycin (79.4 - 100%); penicillin (79.4 - 100%); penicillin (91.4 - 100%) and septrin (76.5 - 91.4%) as shown in Table 1.

The Gram-positive bacterial pathogens implicated in reproductive health infections in this study were mostly susceptible to ceftriazone (97.8 – 100%), ciprofloxacin (88.9 – 100%), rocephin (83.3 – 100%), ciprofloxacin (88.0 - 91.4%), azthromycin (75.0 – 88.9%), zinnat (75.0 - 87.5%) and erythromycin (50.0 – 80.0%); while the most generally resisted antibiotics were ampicillin (66.7 – 94.4%); streptomycin (53.8 – 100%); chloramphenicol (77.8 – 100%), penicillin (72.2 – 100%) and septrin (50.0 – 91.4%). Moderate to high resistance were also exhibited against gentamicin (38.5 – 66.7%) and tetracycline (55.0 – 75.0%) Ofloxacillin was highly susceptible except among the β-haemolytic Streptococcus species (Table 2).

The results of the antifungal assays indicated that the Candida albicans strains were mostly susceptible (84.6 – 100.0%) to the test antifungals, while other Candida strains, Candida glabrata, Candida
Antimicrobials on reproductive infections

Table 1: *In vitro* antibiotic susceptibility profiles of Gram-negative and Gram-positive bacterial pathogens implicated in reproductive health infections

<table>
<thead>
<tr>
<th></th>
<th>Gram-negative</th>
<th>Gram-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>0.0</td>
<td>14.3</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.0</td>
<td>20.6</td>
</tr>
<tr>
<td>Chloramphenico</td>
<td>ND</td>
<td>48.8</td>
</tr>
<tr>
<td>Azthromycin</td>
<td>100.0</td>
<td>92.3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>25.0</td>
<td>57.1</td>
</tr>
<tr>
<td>Perfoxacin</td>
<td>100.0</td>
<td>91.3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50.0</td>
<td>66.7</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.0</td>
<td>8.6</td>
</tr>
<tr>
<td>Rocephine</td>
<td>100.0</td>
<td>97.8</td>
</tr>
<tr>
<td>Zinnat</td>
<td>ND</td>
<td>100.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.0</td>
<td>42.9</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>ND</td>
<td>90.3</td>
</tr>
<tr>
<td>Septrin</td>
<td>ND</td>
<td>23.5</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Fortum</td>
<td>ND</td>
<td>64.3</td>
</tr>
</tbody>
</table>

pseudotropicalis and Candida tropicalis were mostly resistant to the test antifungals (Table 3). However, the mono-resistance exhibited by the Candida species was between 5.0 and 11.1% while the multi resistance was between 88.9 and 95.0%.

Discussion
The microbial pathogens assayed for in this study also overlap with those associated with some sexually transmissible infections (STIs). Gram-negative bacteria for example have previously generated much interest at the University College Hospital, Ibadan, because they have mostly been multiple drug

Table 2: *In vitro* antifungal susceptibility profiles of *Candida* species implicated in reproductive health infections

<table>
<thead>
<tr>
<th></th>
<th>Candida Albicans</th>
<th>Candida Glabrata</th>
<th>Candida Tropicalis</th>
<th>Candida Pseudotropicalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoten tablet</td>
<td>92.3</td>
<td>33.3</td>
<td>33.3</td>
<td>27.5</td>
</tr>
<tr>
<td>Mycoten cream</td>
<td>88.4</td>
<td>33.3</td>
<td>33.3</td>
<td>30.0</td>
</tr>
<tr>
<td>Canesten tablet</td>
<td>88.4</td>
<td>27.8</td>
<td>27.8</td>
<td>27.5</td>
</tr>
<tr>
<td>Canesten cream</td>
<td>88.4</td>
<td>5.6</td>
<td>5.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Fluacam</td>
<td>96.1</td>
<td>0.0</td>
<td>0.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Tetradox</td>
<td>88.4</td>
<td>11.1</td>
<td>11.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Intezol</td>
<td>96.1</td>
<td>33.3</td>
<td>33.3</td>
<td>25.0</td>
</tr>
<tr>
<td>Mycostatine</td>
<td>100.0</td>
<td>38.9</td>
<td>38.9</td>
<td>20.0</td>
</tr>
<tr>
<td>Flagyl</td>
<td>84.6</td>
<td>5.6</td>
<td>5.6</td>
<td>10.0</td>
</tr>
</tbody>
</table>
resistant to many of the available old generation antibiotics like ampicillin, septrin, gentamicin, tetracycline, streptomycin and penicillin (7-10). In this study, the resistance of many of these pathogens to these groups of antibiotics is also noted. This high level antibiotic resistance patterns obtained from a developing country like Nigeria also confirms some earlier reports (11, 12), which similarly noted that the success of ampicillin, tetracycline, streptomycin and gentamicin is currently being threatened by the emergence of resistant, often pathogenic bacteria, and that the bacterial resistance will probably remain the key issue that dictates the future use of such currently marketed antibiotics.

In this present study as well, H. ducreyi strains were highly resistant to ampicillin, streptomycin, gentamicin, penicillin and tetracycline, while G. vaginalis strains were highly resistant to ampicillin, streptomycin, gentamicin, erythromycin, penicillin and tetracycline. The fear therefore, is that spread in antibiotic resistance among pathogens implicated in reproductive health infections can get to alarming increase with time in Nigeria, more especially since surveillance studies are quite low, as well as the relatively high habitual nature of self medications among most of the citizenry.

That the antibacterial properties of the sulfonamides are limited in terms of spectrum of activity and potency, and that bacterial resistance to the sulfonamides is prevalent and continues to restrict the use of many of these agents in current infectious disease chemotherapy had been earlier reported (13). High percentage resistance against septrin in this study further supported this previous report. Klebsiella spp. was found to be highly resistant to ampicillin, streptomycin, chloramphenicol and tetracycline, while E. coli strains were highly resistant to ampicillin, streptomycin, chloramphenicol, tetracycline and septrin. Proteus spp. was also highly resistant to ampicillin, streptomycin, chloramphenicol, gentamicin, erythromycin, tetracycline and septrin. Widespread dissemination of a predominant antibiotic resistance plasmid has been found to occur among E. coli and K. pneumoniae, therefore, use of broad-spectrum oral antibiotics and probably poor infection control practices may also facilitate the spread of plasmid-mediated resistance among these pathogens (14).

Systemic quinolones such as peroxacin, ofloxacin and ciprofloxacin are now available in clinical use and have become important group of antibacterial agents suitable for treatment of bacterial infections (15). Peroxacin was introduced into Nigerian markets around 1999, and has been found to be very effective against most of the common Gram-negative strains in Nigerian environment (10). The third generation cephalosporins such as ceftriaxone (rocepine) were reported to offer the advantage of β-lactamase resistance (13), a characteristic primarily attributed to the acylamino-side chain. This probably explains the higher percentage susceptibility of the pathogens to this group of newer generation antibiotics in the present study.

In some previous studies (10,16,17), in which in vitro activities of commonly available quinolones among bacterial pathogens from various clinical specimens were compared, it was reported that a greater percentage of the pathogens were susceptible to the quinolones. Similarly, the antibiotic susceptibility pattern among Proteus strains in the present study was between 50.0% and 100% towards ofloxacin, peroxacin, ceftriazone, ciprofloxacin and ceftazidine but between 55.0 - 100.0% among the E. coli strains towards ciprofloxacin, ceftazidine, ofloxacin, peroxacin and ceftriazone, while higher susceptibilities were observed among the non-haemolytic Streptococcus spp., β-haemolytic Streptococcus and Staph. aureus towards ofloxacin, rocepine, peroxacin, ceftriazone and ciprofloxacin. These species were however, highly resistant to ampicillin, chloramphenicol, penicillin, streptomycin, gentamicin, tetracycline, streptomycin and septrin.

In spite of the recorded potency of the fluoroquinolones and other newer antibiotics, there are reports that fluoroquinolone-resistant strains are currently being isolated in other countries like Asia and the Western Pacific, and it seems inevitable that there may be increase in other countries (9), especially developing countries like Nigeria, where the fluoroquinolones are currently commonly used for the prevention and treatment of RHIs or other infectious diseases are readily available over-the-counter without prescriptions, therefore, prophylactic or therapeutic self-use of such antimicrobials can serve as a major risk factor for having infections with resistant microbial pathogens as earlier implicated.

In the some previous studies (18, 19), the antifungal susceptibility of strains of Candida species were tested against fluconazole, miconazole, intraconazole, fluconazole, ketoconazole, amphotericin B and fluocytosine, and it was found that most of the
Candida species showed a relatively low MIC values to these antifungal agents. The use of these antifungal drugs were however, found to be very effective against Candida albicans (20, 21), (which has been the most frequently isolated pathogen of the reproductive fungal infections), as well as other non-albicans Candida (22-24). The clinical Candida albicans strains assayed for in this study were also mostly susceptible to the readily available antifungal agents; however, high level of resistance was also recorded among the non-albicans species against the antifungal agents. Most of the non-albicans species were also found to be multi resistant towards the antifungal agents. The indication of this finding is that with time recurrent candidiasis may occur among patients.

The impact of infections of RHIs include major complications, and further, several of such infections singly or in association with STIs can also increase the transmission of HIV infection, and epidemic spread of HIV infection in many parts of the world. For example, if left untreated, gonorrhoea can lead to complications such as pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy or infertility. Infection during pregnancy may lead to spontaneous abortion, stillbirth or neonatal complications. Wiener et al (14) had earlier suggested that antibiotic use and regular survey of antibiotic resistance patterns among pathogens should be monitored and controlled. A very vital point to note however; is that many Nigerians are usually involved in self prescription and self medication as normal habits or due to the major problems of non-adequate primary health care facilities in the country. Therefore, it is important that regular investigations such as this present preliminary study be further followed up, so that comprehensive information can at least be provided as regards the most potent antimicrobials to be used in cases of RHIs. Apart from the few standard primary health care centres or hospitals and some teaching hospitals like the University College Hospital, Ibadan, Nigeria, where this study was carried out, prescriptions are often given to patients without adequate laboratory (antibiotic / antimycotic susceptibility) testing.

Apart from the increasing prevalence of high antibiotic resistant strains, other factors such as efficacy, cost and duration of therapy among others are very important considerations in view of the existing poverty amongst majority of the citizenry. Inadequate or total lack of current and more sophisticated diagnostic facilities and other socio-economic factors are also prevalent as regards the effectiveness of most of the newer antibiotics as previously noted (25). The cost for the complete dose for most of the older generation antibiotics are usually between N 200- N600 naira while the newer generation antibiotics may cost between N 1,500 and N 3, 000 per treatment, meanwhile, the current official minimum wage in Nigeria is about N 6, 500, while some workers earn between N 3,000 and N 4,500 per month. This explains one of the fundamental contributing factors to self medication and common usage of the older generation antibiotics, in spite of the fact that most of them are generally resisted by the bacterial pathogens. Adulteration of clinical drugs is another menace in the country that the drug regulation body in the country, National Agency for Food and Drug Administration and Control (NAFDAC) has been battling for several years.

Some patients would even resort to herbal therapies (26) instead of orthodox treatments based on the level of poverty and inadequate primary health care facilities because there are strong advertisements and peer pressures on the potency of herbal medications. A survey of 4047 [n = 1626 (40.7%) males; n = 2421 (59.3%) female] patients that presented at the Special Treatment Clinic (STC) during this study, as shown in Table 1 indicated that with time, the number of patients presenting at the center diminished. Although medical plants are currently used by rural (and urban) population as herbal remedies for gynecological and reproductive disorders (27, 28), as well as in the treatment of sexually transmitted diseases in women and men (29, 30) but herbal practice in the country currently lacks standardisation in processing, packaging and administration. However, there is increase in the number of patients consulting herbal practitioners. All these are some significant social and population issues affecting public health in the country.

In recent times in Nigeria, advocacy and health support on HIV/AIDS is relatively on the increase; however, infections of non-HIV reproductive health importance are not getting the expected national attention. This research study highlights the laboratory findings that the bacterial pathogens implicated in RHIs were mostly phenotypically resistant to the old generation antibiotics; however, these antibiotics are the most widely available and affordable in Nigeria. The gender issues in which most wives, especially the non-literate wives do not...
get the cooperation of their husbands in joint diagnosis and treatment of reproductive infections, as well as the social stigma sometimes attached to female reproductive infections, leads to self medication practices, involving the use of cheaper antimicrobials. As earlier suggested by Wilkinson et al.[31], there is a need for periodic studies that are more nationally representative as well as those that are more cross sectional, which can be used to monitor the trends in RHI prevalence more reliably, due to the worldwide reports on antibiotic resistance, especially among the enterobacteria (32). Accurate and regular assessments of the effect of antimicrobial agents on the clinical pathogens implicated in RHIs could be of clinical relevance and of public health interest, since underestimation and proper cure of the diseases would lead to cumulative effects of greater concerns, which ultimately may not be limited to the country. It is also necessary for policy makers to develop strategies for monitoring RHIs treated in the private health sectors in Nigeria. The possibility of antibiotic resistance due to adulterated drugs is also of interest and a pilot research on this area is currently going on in our laboratories.

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References