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Antibiotic resistance in *Candida albicans* and *Staphylococcus aureus* involved in vaginitis: Case study of Dschang town, Cameroon

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ABSTRACT

Nowadays, vaginal candidiasis and bacterial vaginosis are frequently encountered in medical practice and antibiotic resistance in implicated pathogens has not been reported in Dschang. This study sought to determine the antimicrobial susceptibility patterns of 198 isolates of *Candida albicans* and 300 strains of *Staphylococcus aureus* which caused vaginal candidiasis and bacterial vaginosis in Dschang in 2003 and 2005 respectively, using a standardized disc diffusion technique. Results showed a significant high resistance ($p<0.05$) to ketoconazole (61.0%), fluconazole (74.7%), amphotericin B (79.3%) and griseofulvin (100%) by *C. albicans*. Sensitivity rates to commonly used antimycotics such as nystatin (100%), clotrimazole (82.3%) and miconazole (73.2%) are still very high, thus, in life threatening infections, treatment with these agents is advocated for empirical therapy. The profile of *S. aureus* heavily favours gentamycin (91.7%), amoxycillin – clavulanic acid (87.7%) and ciprofloxacin (81.3%) ($p<0.05$) which are commonly used. The extent of resistance to erythromycin (70.3%), cloxacillin, (76.6%) and penicillin (81.0%) favours their retirement consideration unless in vitro studies depict otherwise. Vancomycin exhibited 100% efficacy. The present findings highlight the need for a regular review of the antimicrobial susceptibility profiles of commonly isolated organisms in any environment, with a view to ensuring a rational choice for both empirical and definitive antimicrobial chemotherapy.

Keywords: Vaginitis, *Candida albicans*, *Staphylococcus aureus*, antibiotic resistance

INTRODUCTION

Vaginitis is amongst the most prominent infectious diseases encountered in clinical practice today. Knowledge of the aetiology of this infection and the frequency of resistant pathogens in any locality is vital, not only to aid empirical therapy, but also to best minimize the problem of microbial resistance including healthcare costs.

Antibiotic resistance still poses a problem of enormous magnitude in most developing countries particularly because of misuse and overuse of drugs, lack of control measures, easy accessibility and the presence of substandard drugs in the market [1– 4]. Thus, increasing resistance to all drugs is predicted. In view of the above, continuous surveillance of antibiotic profiles in pathogenic organisms should be accorded priority consideration because of the impact on therapy and control of infectious diseases.

This report highlights the antimicrobial susceptibility profiles of *Candida albicans* and *Staphylococcus aureus* associated with vaginal candidiasis and bacterial vaginosis in Dschang. This is with a view to make information available to clinicians to guide patient management and to hopefully develop a practical antibiotic policy which if properly implemented may reverse any untoward effect that antibiotic resistance may cause on the population.

Vaginitis remains a common phenomenon resulting in great discomfort and decline in the health of many women. This problem is often compounded by the fact that it can be caused by several different organisms, sometimes at the same time, as well as by hormonal changes, allergies or irritations [9]. VC and BV are reportedly the most preponderant forms of vaginitis in medicine currently [9-15]. Continuous surveillance of antibiotic profiles of pathogens from various populations is deemed imperative. Such studies are necessary so that proper and tighter antibiotic control policies can be intensified within the hospital and the community to reduce antibiotic selection pressure which leads to high rate of resistant organisms. Furthermore, susceptibility studies enable the observation of changing resistant patterns over time. More still, in

developing countries like Cameroon with limited resources, such investigations will guide rational use of antibiotics. Though the prevalence of microbial resistance to different antimicrobials may vary from one environment to another, it is common knowledge that the situation is progressive and is worst in the hospital environment.

It is understood that, for many decades, pathogenic bacteria and fungi have caused and still continue to cause serious morbidity in humans. Clearly, the number of fungal infections, especially opportunistic fungal infections has risen considerably worldwide. This is largely attributed to the increased use of newer and more effective antibacterial agents and the rapidly expanding population of immuno-suppressed patients. Similarly, many new agents have been introduced to combat such infections in order to curb this disturbing new trend. [7,16]. In consequence, antimicrobial testing has evolved, not only to optimize chemotherapy, but also to observe changing patterns of resistance from year to year and to further evaluate the therapeutic potential of new agents [7, 16].

This study examined *in vitro* resistance to commonly used antimicrobial agents in *C. albicans* and *S. aureus* related to genital infections (VC and BV) in Dschang in 2003 and 2005.

MATERIALS AND METHODS

Pathogens tested:

This study included 198 isolates of *C. albicans* isolated from female patients who presented with vaginal candidiasis (VC) at some healthcare centres in Dschang (The Dschang District Hospital – DDH, Fiangep Polyclinic, Clinique Bienveillance de Foto, Centre de Santé Fometa and Hôpital Saint Vincent de Paul Mission Catholique Sacré – Coeur) from June to December, 2003, while 300 strains of *S. aureus* from cases of bacterial vaginosis at the DDH from January to December, 2005. VC and BV were diagnosed based on presentation of clinical symptoms in patients and laboratory findings (positive direct examination and culture of each cervical swab). A second cervical swab sample from each patient not used for direct examination was cultured on a set of blood agar and Sabouraud Dextrose agar, and on chocolate agar and MacConkey agar. One set of the blood agar plates and the chocolate agar plates was incubated in an atmosphere of enhanced carbon dioxide (5-10%), that is, in a candle extinction jar for 24-48 hours at 37°C. The second set of blood agar plates was incubated anaerobically at 37°C using the GasPak anaerobic jar (BBL anaerobic systems) with a palladium catalyst and a gas generating kit. The MacConkey agar plates were incubated in air at 37°C for 24-48 hours. One set of Sabouraud Dextrose media plates was incubated at 37°C for up to 7 days, whereas the second set of plates for this medium was incubated at room temperature (25-30°C) for up to two weeks (14 days).

The cultural characteristics (macroscopy) of various colonies showing significant growth on the agar media used were studied. Colonies of suspected pathogens were first purified by sub culturing onto fresh media including Nutrient agar and Sabouraud agar before identification. *Staphylococcus aureus* was identified by gram staining, oxidase, catalase and coagulase (slide and tube) tests. Gram staining and germ tube tests were done for *Candida albicans*.

Infections were characterized by vaginal discharge and /or bleeding with one or more of the following clinical manifestations – vaginal soreness, irritation or itching, rashes, warts or maps (white patches), pain or discomfort during sexual intercourse, abdominal pain and burning during urination (dysuria). The fungal and bacterial pathogens tested were isolated from cervical swabs collected from patients suspected with vaginitis as described above. The cervical specimens were processed using standard microbiological methods [5] in the laboratory of Applied Biology and ecology, Department of Animal Biology, University of Dschang. Only one isolate of each type of pathogen was considered per patient since more than one isolate was obtained from some patients who revisited the health centre for control tests, and all isolates tested were clinically significant.

Antimicrobial Susceptibility Testing

Antifungal susceptibility testing was carried out on Sabouraud dextrose agar (Oxoid) using the disc agar diffusion of Bauer *et al.*[6], modified and standardized by the Clinical and Laboratory Standards Institute in 2005 [7] formerly referred to as the National Committee for Clinical Laboratory Standards-NCCLS [8]. Antifungal discs (ABTEK Biological Ltd, Liverpool, UK) with the

following drug contents were used for the study: miconazole (monistat) 10µg, fluconazole (diflucan) 10µg, clotrimazole (mycelex)10µg, ketoconazole (nizoral)10µg, econazole (ecostatin)10µg, nystatin (mycostatin) 10µg, amphotericin B (fungizone) 20µg and griseofulvin (grifluvin)10µg. Antifungal testing was carried out on fresh isolates of *C. albicans* grown on Sabouraud medium at 35°C for 24hours.

Susceptibility testing on *S. aureus* was done on Mueller – Hinton agar (Oxoid) and antimicrobial sensitivity discs for vancomycin (30µg), ampicillin (10µg), gentamicin (10µg), erythromycin (5µg), tetracycline (10µg), chloramphenicol (10µg), penicillin G (I unit), cloxacillin (5µg), streptomycin (10µg), amoxycillin/ clavulanic (30µg) acid and ciprofloxacin (5µg) (ABTEH Biological Ltd, Liverpool, UK) were used. Inocula were prepared from pure fresh isolates grown on nutrient agar at 35°C for 24 hours.

Inocula which contained approximately 10^6 CFU/ ml corresponding to 0.5 McFarland turbidity standard were streaked on to the various sensitivity media using sterile swab sticks. After swabbing, the antimicrobial discs were placed on the surface of inoculated agar plates for 15 minutes and incubated at 37° C for 18 to 24 hours. Growth inhibition zone diameters were measured in millimetres using a calibrated ruler and results were interpreted as sensitive, moderately susceptible or resistant by the method of Bauer *et al* [6], and as recommended by the CLSI[7]

Statistical analysis

Analysis of variance (ANOVA) was carried out to identify differences in means of zone diameters recorded for the various isolates against each drug tested. Various means were then separated using the Student Newman Keul's (SNK) test. Significance level was set at 5%. These analyses were done using SPSS software.

RESULTS

In the antifungal susceptibility testing, all the 198 (100 %) isolates of *C. albicans* were sensitive to nystatin, clotrimazole (82.3 %), miconazole (73.2 %) and econazole (60.6%) were also significantly effective ($p<0.05$). Percentages of susceptibility were below 50 % for ketoconazole, fluconazole and amphotercin B, and the isolates demonstrated significant resistance ($p<0,05$) to these agents including griseofulvin which depicted gross in efficacy against *C. albicans* (Table 1).

All the 300 isolates of *S. aureus* were sensitive to vancomycin. Gentamicin had 91.7 % efficacy, followed by amoxicillin-clavulanic acid (87.7 %), ciprofloxacin (81.3 %) and streptomycin (81.0 %) ($p<0.05$). significant high resistance (> 60 %) was recorded for cloxacillin and penicillin G. Ampiciline, tetracycline, erythromycin and chloramphenicol were also highly ineffective though resistance rates for these agents were insignificant ($p>0.05$) (Table 2).

Table 1: Antifungal Susceptibility Profile of *Candida albicans* associated with vaginitis in Dschang, 2003

Antifungal Agents (Concentration)	Total Number of strains tested	Number (%) Susceptible	Number (%) Moderately Susceptible	Number (%) Resistant
Nystatin(10µg)	198	198(100)^{a}	-	-
Clotrimazole (10µg)	198	163(82.3)^{ab}	26(13.1)^{bc}	9(4.5)^{c}
Miconazole (10µg)	198	145(73.2)^{ab}	39(19.7)^{bc}	14(7.1)^{c}
Econazole (10µg)	198	120(60.6)^{ab}	34(17.2)^{bc}	44(22.2)^{bc}
Ketoconazole(10µg)	198	77(39.0)^{b}	61(30.8)^{b}	60(30.3)^{bc}
Fluconazole(10µg)	198	50(25.3)^{b}	49(24.7)^{b}	99(50.0)^{b}
Amphotericin B(20µg)	198	41(20.7)^{b}	126(63.6)^{a}	31(15.7)^{bc}
Griseofulvin(10µg)	198	-	3(1.5)^{c}	195(98.5)^{a}

Percentages in the same column followed by the same letters are not significantly different ($p>0.05$).

Table 2: Antimicrobial Susceptibility Profile of *Staphylococus aureus* associated with Vaginitis in Dschang, 2005

Antibacterial Agents (Concentration)	Total Number of strains tested	Number (%) Susceptible	Number (%) Moderately Susceptible	Number (%) Resistant
Vancomycin (30µg)	300	300(100)[a]	-	-
Gentamicin(10µg)	300	275(91.7)[a]	15(5.0)[c]	10(3.3)[c]
Amoxycillin – Clavulanic acid (30µg)	300	263(87.7)[a]	20(6.7)[c]	17(5.6)[c]
Ciprofloxacin(5µg)	300	244(81.3)[a]	40(13.3)[bc]	16(5.3)[c]
Streptomycin(10µg)	300	243(81.0)[a]	32(10.7)[bc]	25(8.3)[c]
Ampicillin(10µg)	300	123(41.0)[b]	74(24.7)[b]	103(34.3)[b]
Tetracycline(10µg)	300	107(35.7)[b]	109(36.3)[ab]	84(28.0)[bc]
Chloramphenicol(10µg)	300	106(35.3)[b]	115(38.3)[a]	79(26.3)[bc]
Erythromycin(5µg)	300	89(29.7)[b]	129(43.0)[a]	82(27.3)[bc]
Cloxacillin (5µg)	300	70(23.3)[b]	34(1.3)[c]	196(65.3)[a]
Penicillin G (1 unit)	300	57(19.0)[b]	15(5.0)[c]	228(76.0)[a]

Percentages in the same column followed by the same letters are not significantly different (p>0.05).

DISCUSSION AND CONCLUSION

Results of the present study showed that nystatin was effective against all the yeast isolates tested, suggesting that this drug is not yet abused. Clotrimazole and miconazole were also significantly effective (70% sensitivity) against *C albicans*. In the light of relatively moderate yet significant sensitivity (60%) to econazole, the use of this dug remains favourable. Significant resistance was recorded for ketoconazole (61.0%), fluconazole (74.7%), amphotericin B (79.3%) and griseofulvin (100%). These results are similar to those obtained by Sojakova *et al.* [17] in Slovakia with minimum resistance of *C. albcans* to econazole, clotrimazole and nystatin, and relatively high resistance to ketoconazole, miconazole, itraconazole and fluconazole. One hundred percent susceptibility to nystatin by yeast isolates is also documented by other workers [18]. On the other hand, the present findings are at variance with results obtained in Europe by Pliego Castaneda *et al.* [10] which indicate 100% susceptibility to amphotericin B, 63% to fluconazole and 59% to ketoconazole. Chavanet *et al* [19] in France recorded 100% susceptibility to miconazole and econazole, and 59% to fluconazole for *C. albans* isolated from HIV patients. This study discouraged prolonged azole therapy in severely immuno-compromised patients to avoid emergence of resistant strains.

The first choice for vaginal candidiasis is topical therapy and includes vaginal creams, suppositories or tablets. However, patients have to be thoroughly screened to determine whether systemic drugs are needed in a particular case in order to prevent auto-infection from extra-genital sites [9, 20].

In the present investigation, the result of the anti-bacterial susceptibility showed that gentamicin, augmentin ciprofloxacin and streptomycin had significant in vitro efficacy (>80%) against *S aureus*. High resistance was recorded for ampicillin (59%), tetracycline (64.3%), chloramphenicol (64.6%), erythromycin (70.3%), cloxacillin (76.7%) and penicillin (81.0%). These results are in conformity with the findings of Ahmed *et al.* [20] in Karachi, Groppo et al. [21] in Brazil, and Sattar *et al.* [22] in Islamabad where *S. aureus* is also reported to be highly resistant to penicillin, ampicillin and erythromycin. High resistance to ciprofloxacin (60%) by *S. aureus* is reported in Nigeria [23]. Findings in Kuwait show 100% susceptibility to glycopeptides, 84% resistance to gentamicin and 96% resistance to erythromycin by isolates of *S. aureus* [24]. Reduced susceptibility to glycopeptides by *S. aureus* has been reported [25-28]. Traditional medicine and drug abuse may greatly contribute to drug resistance development in this environment.

The present results show that susceptibility testing is imperative for definitive antibiotic drug therapy in this locality.

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