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BACTERIOLOGICAL AND MYCOLOGICAL CHARACTERIZATION OF SOME PATHOGENS OF THE UROGENITAL TRACT IN BUEA SUBDIVISION (SOUTH WEST REGION CAMEROON).

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ABSTRACT

Introduction/ Urogenital tract infections have a considerable socio-economic impact in Cameroon inparticular and in developing countries in general, as they represent a major cause of morbidity, infertility and increase susceptibility to cancers and HIV/AIDS infections. Yet epidemiological data which are essential in designing a proper strategy to fight against these ailments are rare in Cameroon.

Objective: The main objective of this study is to establish a profile of infectious pathogens associatedwith the urogenital tract from symptomatic cases and to determine the antibiotic susceptibility pattern to commonly used antimicrobial agents.

Method: The isolation of infectious agents of bacterial and fungal origin from urogenital specimens in Buea was evaluated alongside their susceptibility patterns to commonly used antibiotics by the Kirby Bauer method. During a 3-month period, April – June 2007, a total of 220 samples from the urogenital specimens (vaginal, urethral and urine) was collected and analyzed from symptomatic male and female cases attending some health centers in the Buea Health District. All specimens were subjected to biochemical tests for identification and differentiation of individual isolates.

Results: In all, 145 pathogens were isolated; 101 were of bacterial origin while 44 were of fungal (yeast) origin. The prevalence of infectious agents in males [21.3% (17/80)] was different from females [74.30% (104/140)]. Infections were prevalent in individuals within the age group of 20-29, for both sexes, strongly followed by those within the range of 30-39. Ranking the infectivity of the clinical specimens, it was observed that vaginal specimens (70.25%) were the most infected followed by urine (21.49%) and urethral specimens (8.26%) being the least. Bacterial strains were found to be mostly sensitive to tociprofloxacin and frequently resistant to ampicillin and augmentin. On the other hand, yeast strains were mostly sensitive to ketoconazole while flucytosin and amphotericin B were of least sensitivity.

Conclusion: Females were more infected than males and not all the isolates were present in both sexes. The vagina specimens had the highest pathogenic load. *E. coli* was the most predominant isolate from the urinary tract. There was a high prevalence of vaginitis (Candida and Gardnerella vaginalis infections) within the area of study compared to any other infectious agent. Results of antibiotic susceptibility tests revealed that the best drug for treatment of bacterial infections of the urogenital tract was ciprofloxacin, followed by ceftriaxone and amikacin; against ampicillin and augmentin which showed a high degree of resistance to the infectious isolates. On the other hand, the most effective antifungal was ketoconazole, followed by nystatin. The most exhibited multidrug resistant pattern was augmentine-ampicillin-cotrimoxazole. This study revealed that both males and females should be examined from time to time for infection so as to avoid progression to severe states with serious complications.

Key words: Urogenital tract Infectious Agents, Antibiotic Susceptibility Patterns, Buea

RESUME

Introduction: les Infections de l'appareil génito-urinaire ont un impact socio-économique considérable au Cameroun en particulier et dans les pays en développement en général, car ils représentent une cause majeure de morbidité, de stérilités et augmentent la susceptibilité aux cancers et á l'infection au VIH / SIDA. Pourtant, les données épidémiologiques qui sont essentiels dans la conception d'une stratégie adéquate pour lutter contre ces affections sont rares au Cameroun.

But: L'objectif principal de cette étude est de déterminer le profil infectieux associé à l'appareil uro-génital à partir de cas symptomatiques et de déterminer leur susceptibilité aux antibiotiques couramment utilisés.

Méthode: L'isolement d'agents infectieux d'origine bactérienne et fongique à partir d'échantillons urogénitaux à Buea a été évalué de même que leur sensibilité aux antibiotiques par la méthode Bauer Kirby. Au cours d'une période de 3 mois, Avril - Juin 2007, un total de 220 échantillons d'origine uro-génital (vagin, l'urètre et l'urine) ont été recueillies et analysées. Ces échantillons provenaient des individus des 2 sexes ne présentant aucun symptôme et fréquentant certains centres de santé dans la District de Santé de Buéa. Tous les échantillons ont été soumis à des tests biochimiques pour l'identification et la différenciation des isolats.

Résultats: En tout, 145 agents pathogènes ont été isolés; 101 étaient d'origine bactérienne, tandis que 44 étaient des champignons (levure). La prévalence des agents infectieux chez les hommes [21,3% (17/80)] était différente à celle des femmes [74.30% (104/140)]. Ces infections ont été plus répandues chez les individus dans la tranche d'âge de 20-29 ans, pour les deux sexes, suivis par ceux dans la fourchette de 30-39. Concernant le classement de l'infectiosité en fonction des échantillons cliniques, il a été observé que les spécimens vaginaux (70,25%) étaient les plus infectés suivis par l'urine (21,49%) et les spécimens urétraux (8,26%). Les souches bactériennes ont été jugés surtout sensibles à la ciprofloxacine et souvent résistants à l'ampicilline et l'augmentin. D'autre part, des souches de levure ont été principalement sensible à au flucytosin et au kétoconazole alors que l'amphotéricine B était moins sensible.

Conclusion: Les femmes sont plus infectées que les hommes. Les spécimens vaginaux ont la plus grande charge pathogène. *E. coli* était la souche prédominante isolée du tractus urinaire. Il y'avait une forte prévalence de vaginite (Candida et Gardnerella vaginalis) dans la zone d'étude par rapport à tout autre agent infectieux. Les résultats des tests de sensibilité aux antibiotiques ont révélé que le meilleur médicament pour le traitement des infections bactériennes du tractus urogénital est la ciprofloxacine, suivie par la ceftriaxone et l'amikacine, contre l'ampicilline et l'augmentin qui ont révélé un degré élevé de résistance aux isolats infectieux. D'autre part, l'antifongique le plus efficace était le kétoconazole, suivie par la nystatine. Le profil de multi résistance a été plus prononcé pour la combinaison augmentin-ampicilline- cotrimoxazole. Les hommes et les femmes á titre de prévention doivent effectuer un dépistage de ces infections de temps à autre afin d'éviter la progression vers les états sévère avec complications graves.

INTRODUCTION

Urogenital tract infections are among many related factors that affect the broad continuum of reproductive health. They cause many harmful, often irreversible and costly clinical complications such as reproductive health problems, fetal and perinatal health crises (1). The urogenital tract under normal circumstances is a hostile environment for most infectious agents because of its acidity, its normal flora and its mechanical barriers such as mucus and cilia; but upon alteration of these conditions, organisms tend to reproduce and flourish (2).

Urogenital tract infections are the most common causes of morbidity and occasional mortality. Pregnant women are considered the most affected due to their physiological state, followed by teenagers and prostitutes with multiple sexual partners; all with varying prevalence in different populations in developing countries (3, 4). Generally, women tend to be more susceptible to these infections than their male counterparts (5, 6). Diseases commonly encountered in the urogenital tract include urethritis, cystitis or pyelonephritis, prostatitis, vaginitis and bacteria vaginosis.

The main objective in this study is to establish a profile of infectious pathogens associated with the urogenital tract from symptomatic cases and to determine the antibiotic susceptibility pattern to commonly used antimicrobial agents, so as to provide useful data for epidemiological studies. Specific objectives include: possible isolation of bacteria and fungi infectious agents from the urogenital tract, assessment of the prevalence and occurrence of each of these infectious agents in the study area covered in relation to sex and age group, and investigation of the *in vitro* antibiotic susceptibility pattern of these agents.

MATERIALS AND METHODS

The study was a cross sectional study based on laboratory investigations, carried out in some health centers in the Buea Health District (Provincial Hospital Annex Buea, Mount Mary Health Centre and the University of Buea Health Unit). A questionnaire was designed and administered to all participants. It contained demographic data (age, sex, and occupation), clinical symptoms, and risk factors. Knowledge of sexual practices was correlated with sexual behavior to assess the impact of the various parameters on disease prevalence. The genital specimens were inoculated aseptically onto McCarthny bottles, Nutrient, sabouraud and chocolate agar plates, while urine was inoculated on Cystine lactose electrolyte deficient medium, by the aid of a calibrated sterile wire loop. Plates were incubated at temperatures of 37°C for 24-48 hours after which they were examined for colonial characteristics. Gram smears of genital specimens and urine sediment was carried out. Suspicious colonies were sub cultured on nutrient agar plate for isolation of pure colonies, which were presumptively identified based on their morphologic and colony characteristics, and their identity confirmed and characterized by the following tests: oxidase, catalase, coagulase, motility, whiff, Germ tube and the biochemical kit-analytic profile index (API 20E). Antimicrobial susceptibility was performed by the Kirby-Bauer technique, using 10 antibiotics and 5 antifungals.

Statistical analysis:

Statistical package for social science (SPSS) version 11.0 was used to analyze the data. The chi square test was employed and results were considered statistically significant at $p<0.05$.

RESULTS

Upon analysis of questionnaire, all cases of HIV had infections, close to half of those who were negative also had infections while two third of those who did not know their status had infections. The presence of debilitating diseases in individuals exposed them to infections and half of those without any of the diseases were also infected. Multiple numbers of sexual partners, vagina douching and the practice of anal sex predisposes to infection.

Of the 220 specimens obtained and cultured, 53 were from urine (UR), 57 from urethral specimen (US) and 110 from vagina specimen (VS). 121 isolates were infectious, giving an overall prevalence of 55.0% (121/220). Of all the specimens analyzed, VS accounted for 70.25% (85/121) of infectious agents compared to UR and US which yielded 21.49% (26/121) and 8.26% (10/121) respectively (Table 1).

Table 1: Prevalence of infectious agents in different specimens.

Specimen	Number of positive cases	Total
US	**10 (8.26)***	**57**
VS	**85 (70.25)**	**110**
UR	**26 (21.49)**	**53**
Total	**121**	**220**

US: urethral specimen, VS: vaginal specimen, UR: urine, *Figures in parentheses are in percentages.

The prevalence of infectious agents in males (21.3% (17/80)) was different from that in females (74.30% (104/140)) (Table 2). No significant difference was observed in the prevalence of infection as regards age ($p>0.05$) between the male and female population. Prevalence of infection was highest within the age interval of 20-29 yrs and lowest in those aged over 50 yrs (Figure 1).

Of the 167 genital specimens sampled, 50.90% (85/167) were found to be infectious from females compared to 6.0% (10/167) from their male counterpart (Table 3), giving an overall prevalence of 56.89% (95/167) in all the genital specimens.

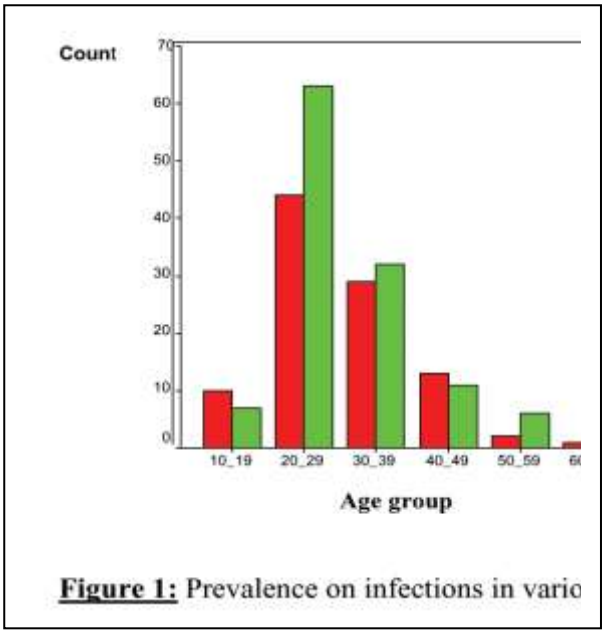

Figure 1: Prevalence on infections in vario

In females the genital tract infectious with the highest prevalence were *Gardnerella vaginalis* (n=37), and *Candida albicans* (n=34); others present included *Citrobacter freundii*, *Proteus mirabilis, Enterobacter aerogenes, K. ozaenae* and *N. gonorrhoea* (where n=1), *Streptococcus sp* (n=8), *Candida sp* (n=7), *K. pneumoniae* (n=5) and *Enterobacter cloacae* (n=2). No *Pseudomonas sp*, *Staphylococcus sp* and *K. oxytoca* were found (n=0).

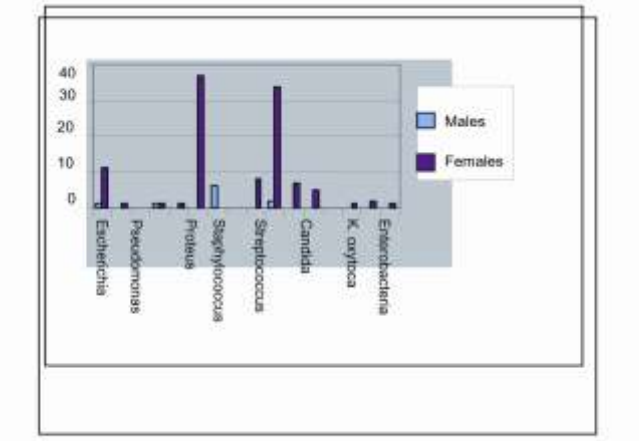

Figure 2: Distribution of infectious agents from genital specimens in both sexe

On the other hand, the most common infectious agents in males were *Staphylococcus aureus* (n=6) and *Candida albicans* (n=2), the least being *Escherichia coli* and *N. gonorrhea* (where n=1). The rest of infectious agents not listed were not found (Fig. 2). From the 53 urine specimens sampled, 26 were infectious, yielding a prevalence of 49.06% (26/53).The most prevalent pathogen was *E. coli* (females n=5, males n=3), followed by *K. pneumoniae (females n=4, males n=2),* and *P. aeruginosa* (females n=3, males n=0), the least being *P. mirabilis (n=1 for both sexes)* and *C. albicans* (females n=1, males n=0). The rest of the infectious agents not listed where not found in urine (figure 3).

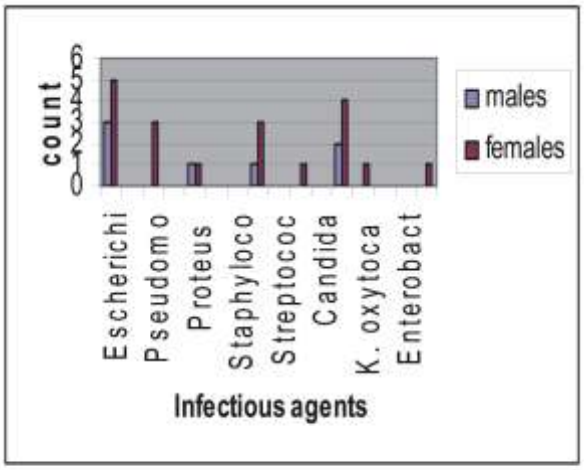

Figure 3: Distribution of infectious agents from urine specimens in both sexes

Table 2: Prevalence of infectious agents in different sexes

Sex	positives	Total
Males	**17 (21.30)***	**80**
Females	**104 (74.30)**	**140**
Total	**121**	**220**

* Figures in parentheses are in percentages. Chi square: x^2= 57.86 df= 1 p= 0.000

All cases of co-infection were seen in female vaginal specimens, and this was common between *Gardnerella vaginalis* and *Candida albicans*. Others were *E. coli* and *Candida sp*, *G. vaginalis, E. coli* and *Candida sp* and finally *P. mirabilis, G. vaginalis* and *Candida* sp (Table 4). Of the 10 antibiotics used for susceptibility testing, resistance came mostly from ampicillin and augmentine, followed by co-trimoxazole, erythromycin, ofloxacin, ciprofloxacin, ceftriaxone, gentamicin, amikacin and cefotaxime in order of decreasing resistance.

Sensitivity was registered in ciprofloxacin, amikacin, ceftriaxone gentamicin, cefotaxime, ofloxacin, co-trimoxazole, erythromycin, ampicillin and augmentine in order of decreasing sensitivity. Cefotaxime, gentamicin, ceftriaxone, ofloxacin, ciprofloxacin, amikacin, erythromycin, co-trimoxazole and augmentine had intermediate sensitivity in decreasing order of sensitivity (Table 5a).

The susceptibility patterns of the infectious agents revealed that the most sensitive was *E. coli*, as shown in Table 5b.

Resistance pattern for yeast infection revealed that strains were mostly resistant to amphotericin B, followed by flucytocin, miconazole, nystatin and finally ketoconazole (Table 6).

DISCUSSION

In light of the study, the distribution of pathogens in the anatomical sites of humans was as follows: VS > UR > US (Table 1). Infections in females accounted for 74.3% compared to 21.3% in males. There was a significant difference between sexes in terms of infection. This may be due to differences in the anatomy of their urogenital structures. Apart from the differences in distance the infectious agents may have to travel, the urethra is located near the rectum in women, so bacteria from the rectum may easily travel up the urethra and cause infections (5). Sexual activities may also cause urinary tract infections in women because bacteria can be pushed into the urethra (6). Using a diaphragm can lead to infections because diaphragms push against the urethra and make it harder to completely empty the bladder. The urine that stays in the bladder is more likely to grow bacteria and cause infections (7).

Infections were prevalent in individuals within the age group of 20-29, for both sexes, strongly followed by those within the range of 30-39. This is accounted for by the increase sexual activity or libido of these youngsters. It was noted that those with multiple sexual partners were victims of infections. Conversely, individuals within the early (10-19) and late (50-59,≥ 60) ages where less infected and those who recorded infections for the old could be due to their decreased immunity to infection, meanwhile for the young could be hygienic malpractices or even sexual activity. There was a significant difference between the age groups ($p>0.05$).

Within the female population, *Candida albicans* and *Gardnerella vaginalis* were more numerous than other infectious agents. Suppression of the normal flora of the vagina leads to overgrowth of this yeast in the vagina and on the labia. This condition is referred to as candidiasis. Many yeast infections occur after the use of a broad spectrum antibiotic. The antibiotic reduces the *Lactobacillus* population in the vagina allowing the pH to rise and the amount of glucose available for *Candida* growth to increase. Due tothe fact that *Candida* is not a bacterium, it is not affected by the antimicrobial (8). Among all the infected cases, co-infection between *Candida species* and *G. vaginalis* recorded 13.6%. Other co-infections were seen with *E. coli* and *Candida species* as well as *E. coli* and *G. vaginalis.* Streptococcal infections were more prevalent among pregnant women and this is in accord with the findings that these bacteria are mostly encountered in women with this physiological status (9). *Neisseria gonorrhoeae* was found to have equal chances of growth in both sexes and isolation was very rare for this species. The other organisms that were isolated such as *P. mirabilis, K. oxytoca, E. cloacae* have been involved in urinary tract infections (10, 11).

From urine specimen, the frequency and order of isolates ties with previous studies, where *E. coli* was always higher than the rest of the infectious pathogens. The trend continues with *Klebsiella sp, Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* (12, 13, 5). Women had more infections (73.08%) than males (26.92%). Fungal infections of the urinary tract primarily affect the bladder and kidneys. Lower UTI with *Candida* usually occurs with urinary catheters, typically after bacteriuria and antibiotic therapy, although candidal and bacterial infections frequently occur simultaneously. *C. albicans* prostatitis occurs infrequently in patients with diabetes, usually after instrumentation (5). The fungi isolate (*C. albicans*) obtained from urine were obtained from a catheter culture and all who used catheters were infected. Antimicrobial usage is considered the most important factor promoting the emergence, selection and dissemination of antimicrobial-resistant microorganisms (14). This study also determined the sensitivity of isolates to various antibiotics commonly used. From the results of the antibiogram, ciprofloxacin (fluoroquinolone) and ceftriaxone (cephalosporin) were the most sensitive drugs for bacteria infections recording a total of 57% and 52.38% sensitivity in all the various isolates respectively. Amongst Gram negative bacteria, they had a greater percentage of 99% and 97% respectively, meaning that they are drug of choice for the treatment of Gram negative bacilli-causing infections. Ceftriaxone was most sensitive for *N. gonorrhoea;* this observation was in accordance with that of Cheesbrough (11). The intensive use of quinolones in the treatment of common infections has led to the spread of resistant microorganisms (15). Ofloxacin which was previously considered as a drug of choice some few years ago showed poor sensitivity pattern to all the isolates (34.92%), but towards Gram negative organisms had a good or high rate of sensitivity (95%); being coherent with previous studies. Amikacin also showed a high percentage of sensitivity (52.38%) to all the infectious agents. Based on studies carried out in Cameroon (Yaoundé), Koulla-Shiro and Abong-Bwemda in 1995 reported *Enterobacter* and *Klesiella* to have low sensitivity patterns, and *Proteus* and E. *coli* a relatively high susceptibility rate (16).

Among the various isolates, *E. coli* was most sensitive, the least being Klebsiella *sp*. The cocci were also resistant, especially *S. aureus, N. gonorrhoea, Streptococcus sp* and finally *S. saprophyticus*. Amongst all the various antifungals employed, ketoconazole showed a sensitivity of 50%, compared to the others. This was followed by nystatin and the other azole (miconazole), flucytosin and finally amphotericin B. *C. albicans* was more sensitive than the other *Candida species*.

CONCLUSION

Females were more infected than males and not all the isolates were present in both sexes. The vagina specimens have the highest pathogenic load. *E. coli* was the most predominant isolate from the urinary tract. There was a high prevalence of vaginitis (*Candida*- 16.3% and *Gardnerella vaginalis*- 17.6% infections) within the area of study compared to any other infectious agent. There was poor knowledge and poor hygienic practices concerning reproductive health (urogenital tract infection acquisition/prevention) within the studied population. Results of antibiotic susceptibility tests revealed that the best drug for treatment of bacterial infections of the urogenital tract was ciprofloxacin, followed by ceftriaxone and amikacin; against ampicillin and augmentin which showed a high degree of resistance to the infectious isolates. On the other hand, the most effective antifungal was ketoconazole, followed by nystatin. The most exhibited multidrug resistant pattern was augmentineampicillin- cotrimoxazole.

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Table 3: Prevalence of infectious agents of genital specimens in different sexes

Sexes	Number of positives	Total
Males	**10 (6.0)***	**57**
Females	**85 (50.90)**	**110**
Total	**95 (56.90)**	**167**

* Figures in parentheses are in percentages.

Table 4: Distribution of Co-infectious agents in female (vaginal specimen)

Co- infectious agents	positives
Gardnerella vaginalis* and *Candida albicans	13
E. coli* and *Candida sp	3
G. vaginalis, E. coli* and *Candida sp	2
P. mirabilis, G. vaginalis* and *Candida sp	3

Table 5a: Antibiotic susceptibility distribution to different antibiotic discs

Antibiotics	Resistant	Sensitive	Intermediate
Ampicillin	49 (77.8)*	14 (22.22)	0 (0.00)
Augmentin	47 (74.60)	14 (22.22)	2 (3.17)
Ceftriaxone	24 (38.10)	33 (50.79)	7 (11.11)
Erythromycin	41 (65.09)	20 (31.75)	2 (3.17)
Cefotaxime	24 (38.10)	32 (50.79)	7 (11.11)
Gentamicin	25 (38.10)	32 (50.79)	6 (9.52)
Ciprofloxacin	24 (38.10)	36 (57.14)	3 (4.76)
Ofloxacin	37 (58.73)	22 (34.92)	4 (6.35)
Amikacin	28 (44.44)	33 (52.38)	2 (3.17)
Co-trimoxazole	39 (61.90)	22 (34.92)	2 (3.17)

- The figures in parentheses are in percentage.

Table 5b: susceptibility pattern of isolates of the bacteria profile

Infectious agents	**Sensitive**	**Resistant**	**Intermediate**
E. coli	135 (67.50)*	55 (27.50)	10 (5.0)
K. pneumonia	23 (20.91)	84 (76.36)	3 (2.73)
P. aeruginosa	8 (26.67)	22 (73.33)	0 (0)
N. gonorrhea	7 (35.00)	13 (65.00)	0 (0)
P. mirabilis	10 (33.33)	20 (66.67)	0 (0)
S. aureus	14 (23.33)	46 (76.67)	0 (0)
Streptococcus sp	23 (28.75)	45 (56.25)	14 (15.00)
K. oxytoca	1 (10.00)	9 (90.00)	0 (0.00)
K. ozaenae	4 (40.00)	6 (60.00)	0 (0.00)
C. freundii	5 (50.00)	4 (40.00)	1 (10.00)
E. clocae	9 (45.00)	11 (55.00)	0 (0.00)
E. aerogenes	5 (50.00)	4 (40.00)	1 (10.00)
S. saprophyticus	14 (35.00)	21 (52.50)	5 (12.5)

Table 6: Antifungal susceptibility distribution

Antifungal	Resistant	Sensitive	Intermediate
Amphotericin (20µg)	32 (72.73)	12 (27.27)	0 (0.0)
Miconazole (10µg)	29 (65.91)	13 (29.55)	2 (4.55)
Nystatin (100UI)	21 (47.73)	21 (47.73)	2 (4.55)
Flucytosin (1µg)	30 (68.18)	12 (27.27)	2 (4.55)
Ketoconazole (10µg)	22 (50.0)	22 (50.0)	0 (0.0)