ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *SALMONELLA TYPHI* AND *CANDIDA ALBICANS* ISOLATES IN DAR ES SALAAM, TANZANIA.

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Summary

The aim of this study was to determine antimicrobial susceptibility testing patterns of *Candida albicans* and *Salmonella typhi* isolates. Fifteen isolates of each microorganism were collected from three hospitals located in Dar es Salaam region within a 3-month period in the year 2005. *Candida albicans* and *Salmonella typhi* isolates were purified by sub-culturing on appropriate culture media in order to obtain pure cultures of the assayed microorganisms. *Candida albicans* isolates were identified by Gram staining technique and germ tube test, while *S. typhi* isolates were also identified by Gram staining technique followed by sub-culturing in various selective and differential culture media, then confirmed by immunological (agglutination) test. In-vitro antimicrobial susceptibility patterns of the assayed microbial isolates were determined by the disk diffusion technique of Stokes. The disk strength and tentative sizes of zones of inhibition (ZI) were interpreted in accordance with American National Committee for Clinical Laboratory Standard (NCCLS). Antifungal susceptibility patterns for *C. albicans* isolates showed that azoles are more efficacious than other assayed antifungal agents. Results of antibacterial susceptibility revealed that all the assayed *S. typhi* isolates were resistant to chloramphenicol and co-trimoxazole, but were sensitive to ciprofloxacin, gentamicin, ampicillin, doxycycline and ceftriaxone. Therefore, this study finding calls for a need to review the current prescription and dispensing practices of antimicrobial agents in both hospitals and pharmacies respectively. Furthermore, it is recommended that a nationwide study on antimicrobial susceptibility pattern should be conducted in order to come up with national policy on rational use of antibiotics.

Key words: Susceptibility patterns testing, antimicrobial agents.

Introduction

Candida species are ubiquitous fungi and they are the most common fungal pathogens affecting humans. *Candida albicans* is responsible for most candidal infections, although non-*albicans* species are encountered with increasing frequency.[1-3] Candidiasis usually affects a wide variety of organs in immunocompetent persons; any warm, moist part of the body exposed to the environment is susceptible to infection. Some of the typical Candidal infections include vaginitis, vulvular rash, oral thrush, diaper rash and infections of nails, rectum and other skin folds.[4] Most forms of *Candida* species affect males and females equally. However, several women worldwide continue to suffer from vulvo-vaginitis candidiasis (VC), which in many populations is second only to anaerobic bacterial vaginosis.[5] The rise in the incidence of VC has been probably attributed to a myriad of factors such as increased prevalence of HIV infection, extensive use of broad-spectrum antibiotics, oral contraceptives, and corticosteroids and other immunosuppressive drugs.[6]

The most commonly used antifungal agents against candidiasis are amphotericin B, fluconazole, ketaconazole, and nystatin. But there are few reports on amphotericin B resistance, particularly on immunocompromised and cancer patients.[7-8] As azole antifungal agents have become important in treatment of mucosal candidiasis in AIDS patients, reports on resistance have also increased.[9-10] Moreover, azoles resistance has also been manifested in non-HIV positive patients and occasionally in individuals not previously exposed to antifungal agents.[11]

Salmonella typhi causes typhoid fever, which is a severe multisystemic illness characterized by the classic prolonged fever, sustained bacteraemia without endothelial or endocardial involvement, and bacterial invasion of and multiplication within the mononuclear phagocytic cells of the liver. The latter is the most prevalent in underdeveloped countries with resources too limited to prevent or treat it.[12-13] *S. typhi* infects only humans, and human transmission occurs through consumption of contaminated food and beverages handled by persons shedding *S. typhi* from stools (or less commonly urine) or water from sewage contaminated with *S. typhi*. For that reason, typhoid fever is still a major public health problem in many developing countries like Tanzania, due to extremely low standard of living of majority of the population with all associated risk factors for the spread of communicable diseases. Consequently, typhoid fever has a considerable negative impact in developing countries because most patients require several weeks of recovery.[14] Antibiotics that form the mainstay of typhoid fever therapy in most of the developing countries are chloramphenicol, ampicillin and cotrimoxazole. However, multidrug-resistant (MDR) strains of *S. typhi* have been reported in various countries.[15-17]

Several methods have been employed in antimicrobial susceptibility testing of fungi (yeast) and bacteria. Some of these methods include disk diffusion, agar dilution methods, broth macrodilution and microdilution, colorimetric microdilution and flow cytometry.[18] Guidelines for the methodology and interpretation of antifungal susceptibility

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testing of *Candida* species were adopted in order to compare broth macro-dilution method (the reference standard) with other method such as disk diffusion and agar dilution methods [19], which are feasible in our setups. This has made it possible for the clinical microbiology laboratory to perform reliable *in vitro* antifungal susceptibility tests on a wide range of yeasts and moulds.

For routine antimicrobial susceptibility testing, two methods have been usually employed, namely liquid medium dilution (LMD) method that is quantitative, and disk diffusion method, which is qualitative or semi-quantitative. Disk diffusion method allows determination of the antimicrobial susceptibility patterns of bacterial/fungal isolates by adjusting the microbial suspensions to that of McFarland 0.5 standard, which is an indicative of the standard suspension density.[20] However, LMD method is expensive and time-consuming test. The LMD method may only be used when few microorganisms are to be tested or during the determination of a very accurate minimum inhibitory concentration (MIC). Therefore, this study intends to assess the antimicrobial susceptibility patterns of *S. typhi* and *C. albicans* isolates by employing the disk diffusion method of Stokes.

Materials and Methods

Collection of biological materials

Fifteen isolates of each of the following microorganisms: *C. albicans* and *S. typhi* were collected from three different health centres viz. Muhimbili National Hospital (MNH), Regency and TMJ hospitals within a 3-month period in the year 2005. *Salmonella typhi* isolates were obtained from recent faecal specimens from 15 patients with typhoid fever, while *C. albicans* isolates were obtained from another 15 patients with candida oral thrush

Purification and culturing

The collected microbial isolates were inoculated in fluid media (peptone water) and incubated for 24 hours to achieve sufficient microbial growth. Then each isolate was sub-cultured in appropriate solid media (for *C. albicans* in Sabouraud's dextrose agar-SDA and for *Salmonella typhi* in Samonella-shigella agar-SSA), in order to obtain pure culture of microorganisms from each isolate.

Candida albicans isolates were identified by Gram staining technique and germ tube test as previously described[20], *Salmonella typhi* isolates were also identified by Gram staining technique followed by sub-culturing in various selective and differential culture media namely MacConkey with salt, Salmonella-Shigella, Kligler iron agars, sulphide indole medium, peptone water and finally confirmed by immunological (agglutination) test using EUROPATH salmonella antigen suspension (Chemoquip, Kenya).

Preparation of antifungal and antibacterial disks

Using Whatman® qualitative filter paper (Maidstone, UK), 5mm disks were punched. For each antifungal/antibacterial agent distinct dilutions were prepared to obtain varying drug concentrations. The assayed antifungal agents were clotrimazole (FDC, India), ketaconazole (Remedica, Cyprus), terbinafine (Cipla, India), griseofluvin and nystatin (Alphaceuticals,UK), and fluconazole (Sedico, Egypt). While for antibacterials were ampicillin and cefuroxime (Medochemies, Cyprus), co-trimoxazole (Elys-Chemicals, Kenya), gentamicin (Wockhardt-Life Sciences, India), ciprofloxacin (Pharmathen-SA, Greece), ceftriaxone (Bristol-Myers Squibb, USA), chloramphenicol, doxycycline and erythromycin (Indico remedies, India).

Analysis of antimicrobial susceptibility patterns

C. albicans and *S. typhi* inoculum were separately prepared by suspending a single isolate colony of each microorganism in about 5ml of Ringer lactate solution and shaken well to break up all clumps of microbial colony/growth. The inoculum's density was estimated visually to match the turbidity of McFarland 0.5 standard [20]. One millilitre of the above prepared suspension was poured onto SDA/Iso-sensitester agar plates, then spread evenly to ensure confluent growth of the microorganisms. The plates were left to dry for 15 minutes allowing the medium to absorb the moisture from the inoculum. Then using a pair of flamed sterilized forceps, appropriate disks containing the antifungal/antibacterial agent to be tested were placed onto the surface of the inoculated agar plate and pressed lightly to ensure complete contact with the agar. The inoculated plates were inverted and incubated at 37°C in 3-5% CO_2 for 20-24 hours. The disk-diffusion method of Stokes[22] was used in this study by employing *C. albicans* (ATCC 90028) and *Escherichia coli* (ATCC 25922) strains as standard microorganisms for the isolates of *C. albicans* and *S. typhi* respectively. The results were noted based on observation of zones of inhibition (ZI) after overnight incubation at 37°C. Sizes of ZI were interpreted as previously described[20-21] and by referring to standard zone diameter for antibacterial/antifungal susceptibility [19]. Then the isolates were noted as susceptible (S), intermediate (I) or resistant (R) for each of the assayed drugs. Each of these assays was performed twice in triplicate for statistical purpose.

Ethical issues

These were addressed by requesting permissions from the respective hospital authorities as well as by both written and verbal personal consents from patients.

Results

A total of 30 microbial isolates (*Candida albicans* and *Salmonella typhi*) were assayed. Different drug concentrations were tested on each microorganism with reference to the standard microorganisms by use of Stokes' disk-diffusion method. Table 1 summarizes the antifungal susceptibility testing patterns of *C. albicans* isolates. Out of 15, only 3 isolates of *C. albicans* were resistant to nystatin (8-12 μg) and griseofulvin (5-10μg). Only one isolate (S/N

9) was resistant to fluconazole (5-25μg), while isolate (S/N 14) was only resistant to fluconazole (5μg). Terbinafine was not effective at 4 μg; however 11 out of 15 isolates that is 73.33%, ($p < 0.001$) were sensitive to the drug at a concentration of 12μg. As it was expected, co-trimoxazole (25-35μg) was not effective against all studied isolates of *C. albicans*. Table 2 shows the patterns of antibacterial susceptibility of *S. typhi* isolates. All isolates were neither susceptible to chloramphenicol (30μg) nor to co-trimoxazole (25-35μg). While only 3 out of 15 isolates (20%) were sensitive to cefuroxime (30μg). On the other hand, all 15 *S. typhi* isolates were susceptible to ciprofloxacin (5μg) and ceftriaxone (30μg), with MIC for ciprofloxacin < 1μg/ml and for ceftriaxone MIC < 0.25μg/ml. Similarly all *S. typhi* isolates were also susceptible to gentamicin (10μg), ampicillin (10μg), and doxycycline (30μg), while manifested intermediate susceptibility to erythromycin (15μg), but they were resistant to cefuroxime (30μg).

Table 1. Patterns of antibacterial susceptibility testing of 15 isolates of *Candida albicans* obtained from 3 different hospitals in Dar es Salaam

C. Albicans isolate S/N	Mean inhibition zone diameter (mm)														
	NYT		CLO		KET		GRS		FLU			TEB		COT	
	12µg	8µg	15µg	3µg	15µg	3µg	5µg	10µg	25µg	15µg	5µg	4µg	12µg	25µg	35µg
1	13	10	29	25	32	27	-	-	17	13	11	-	17	-	-
2	12	9	20	18	23	20			22	15	8	5	19		
3	12	10	21	20	23	18	-	-	23	14	8	-	16	-	-
4	10	8	30	24	32	26	-	-	26	17	10	-	18	-	-
5	10	10	27	18	30	22	-	-	17	13	9	-	18	-	-
6	12	8	28	21	30	23	-	-	19	12	7	-	19	-	-
7	9	9	24	17	25	19	-	-	27	18	8	-	17	-	-
8	8	8	32	24	32	28	-	-	19	13	7	-	16	-	-
9	-	-	12	9	15	12	-	-	-	-	-	-	-	-	-
10	15	12	24	15	25	21	-	-	13	10	7	-	-	-	-
11	-	-	15	13	15	12	-	-	13	10	8	-	-	-	-
12	12	9	29	25	30	27	-	-	31	20	11	-	14	-	-
13	9	8	29	26	32	28	-	-	29	20	9	-	17	-	-
14	-	-	12	10	14	12	-	-	13	10	-	-	-	-	-
15	9	7	30	24	31	25	-	-	30	19	11	-	14	-	-

NYT, nyastatin; CLO, Clotrimazole; KET, ketaconozole; GRS, griseofulvin; FLU, fluconazole, TEB, terbinafine; COT, co-trimoxazole

Table 2. Patterns of antibacterial susceptibility testing of 15 isolates of *Salmonella typhi* obtained from 3 different hospitals in Dar es Salaam

C. Albicans isolate S/N	Mean inhibition zone diameter (mm)															
	ERT	CEF	CHL	CIP			CEFT		AMP	GEN			COT		DOX	
	15µg	30µg	30µg	10µg	5µg	2µg	30µg	10µg	10µg	10µg	5µg	2µg	25µg	35µg	30µg	20µg
1	21	-	-	50	40	35	40	28	27	26	23	20	-	-	30	26
2	18	32	-	48	40	37	36	28	28	24	24	24	-	-	32	26
3	18	24	-	52	40	40	40	28	29	28	24	23	-	-	34	26
4	18	10	-	50	38	39	38	30	25	27	26	26	-	-	34	24
5	22	-	-	47	42	40	40	28	28	26	24	24	-	-	34	28
6	18	-	-	50	44	43	38	28	26	28	24	24	-	-	34	26
7	18	-	-	52	44	44	40	30	28	30	22	23	-	-	34	26
8	19	-	-	46	40	39	36	28	25	30	23	22	-	-	32	27
9	17	-	-	44	38	37	36	29	24	30	22	22	-	-	36	27
10	20	-	-	45	37	37	35	27	26	27	24	25	-	-	34	25
11	18	-	-	48	42	40	42	30	24	28	24	24	-	-	29	24
12	20	-	-	50	39	40	40	28	25	28	26	22	-	-	32	25
13	17	-	-	47	40	39	39	27	27	28	27	23	-	-	30	26
14	18	-	-	39	38	36	36	28	28	27	26	22	-	-	34	28
15	17	-	-	45	36	35	37	28	28	28	25	24	-	-	32	26

ERT, erythromycin; CEF, cefuroxime; CHL, chloramphenicol; CIP; ciprofloxacin; CEFT, ceftriaxone, AMP, ampicillin; GEN, gentamicin; COT, co-trimoxazole; DOX, doxycycline.

Discussion

This study intended to investigate the antimicrobial sensitivity and susceptibility patterns of *C. albicans* and *S. typhi* isolates, and to reveal any possible drug resistance to the routinely used antimicrobial agents against these microorganisms. Results show that antibiotic susceptibility of *S. typhi* isolates manifested similar drug resistance patterns for chloramphenicol (30 μg) and co-trimoxazole (25μg), which had no zones of inhibition (ZI), which means they are not effective against *S. typhi*. Nevertheless, because of their lower cost, they are still used in other areas where local strains are sensitive.[22] These study findings are in agreement with other studies that have also reported drug-resistant typhoid fever, in which studied isolates were resistant to chloramphenicol, tetracycline, cotrimoxazole and kanamycin.[17, 23-24]

All fifteen isolates of *S. typhi* were susceptible to ciprofloxacin (5μg) with MIC < 1μg/ml and ceftriaxone, but reduced susceptibility with high MIC > 0.5mg/ml of ciprofloxacin has also been reported.[24] Apart from the fact that gentamicin, tetracycline, as well as the first and second-generation cephalosporins are routinely used for determination of resistance to *S. typhi,* but they are not clinically useful for treatment of typhoid fever.[17] However, our results revealed that they have better *in vitro* antibacterial (*S. typhi)* effect compared to chloramphenicol and co-trimoxazole. A third-generation cephalosporin could be the drugs of choice if quinolones (also still effective against *S. typhi*) are to be avoided, due to concerns about quinolone-induced arthropathy and cartilage damage in children.[25]

The overall antifungal susceptibility testing patterns of *C. albicans* showed that azoles were more effective than that of other antifungal agents. Reduced sensitivity/resistance to nystatin and griseofulvin should not be taken lightly, since are some of the antifungal agents that are frequently used by most of the Tanzanians, probably due to their lower prices compared to other antifungals.[26] The observed "resistance" to fluconazole and terbinafine is not unusual, since other authors have also observed similar findings.[27-32] Additionally, co-trimoxazole was also included in antifungal susceptibility testing out of curiosity, in order to verify any effect on *C. albicans*, regardless of its different mechanism of action from that of antifungal agents.[33] Some of the factors that may attribute to the current prevalence of drug resistance are excessive use of antibiotics for treatment of minor upper respiratory tract infections, AIDS related infections, promotional activities, lack of knowledge on drug use and incompliance to medication regimen, just to cite a few.[34] Furthermore, in spite of tirelessly elaborating the study's objectives to the patients, still some were reluctant to participate fearing that the study could have been aimed at analyzing their immunological status. This was aggravated by a very limited time-interval (3 months only) allocated for the entire study. All these call for further investigation into the antimicrobial susceptibility patterns nation-wise.

Conclusion

This preliminary study finding revealed that chloramphenicol and cotrimoxazole besides being documented as the first line antibiotics in treatment of typhoid fever, they are no longer effective against *S. typhi.* Therefore, they should certainly not be indicated for treatment of typhoid fever. While the susceptibility of *S. typhi* isolates to ciprofloxacin and ceftriaxone implies that these drugs may be a good alternative in treatment of increasingly widespread drug resistant typhoid fever. The observed declined sensitivity/resistance of *C. albicans* to nystatin, which might have been attributed to self-medication or/and over prescription of the drug needs to be further investigated. Therefore it is recommended that practices should be reviewed, since most of the commonly indicated drugs for typhoid fever have been found to be ineffective. Finally, we suggest a more extensive and nationwide study on antimicrobial susceptibility testing patterns that will involve a larger sample size, in order to come up with the national policy on rational antibiotics use. In the mean time we suggest that the government through the Ministry of Health and Social-welfare should take immediate strategies to minimize the problem of drug resistance development, and the Tanzania Food and Drugs Authority (TFDA) should adopt some drastic measures to control the use and sale of antimicrobial agents in drug stores and pharmacies.

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