ASSESSMENT OF COMMONLY AVAILABLE ANTIMICROBIAL AGENTS. A STUDY FROM ILALA-TANZANIA.

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ABSTRACT

OBJECTIVE

Microbiological assessment of commonly available antimicrobial agents in Ilala Municipality.

METHODOLOGY

The disc diffusion method was used for the determination of antimicrobial activities.

RESULTS

Amoxicillin exhibited ZI between 21mm (Elys) and 23mm (Zenufa) against *E. coli,* and between 21mm and 23mm (Elys) against *S. aureus. A*mpicillin samples yielded ZI from 20mm (India) to 25mm (Keko) against both bacteria. Dicloxacillin exhibited ZI between 13mm (Keko) and 16mm (India, Keko) against *E. coli and* from 15mm (Keko) to 18mm (Shelys) against *S.aureus.* Ciprofloxacin samples (India) exhibited ZI between 22mm and 25mm against both bacteria.

On the other hand ketoconazole exhibited ZI between 16.5 and 19.0 mm against both *Candida albicans* and *Cryptoccocus neoformans.* Nystatin (Cyprus) produced ZI between 10 and 12mm against both fungi; similarly Fluconazole (India) yielded between 16.5 and 20mm of ZI against both *C. albicans* and *C. neoformans*.

CONCLUSION

The antimicrobial agents analyzed in this study have demonstrated substantial antimicrobial activities against the test microorganisms, an indicative of possession of active ingredients.

As far as the *in vitro* microbiological assays are concerned, the study's findings could not reveal any counterfeit drug.

However, further studies should be conducted to confirm the content specifications and other relevant parameters of each pharmaceutical preparation.

INTRODUCTION

Antimicrobial sensitivity tests are performed to test on the effectiveness of antimicrobial agents against microorganisms. Some of the most common methods include serial dilution, ditch plate, cup plate and solid dilution. For the disc diffusion method, the drug inhibits the growth of microorganisms in the area around the disc across which the drug diffuses from the impregnated disc. The inhibition effect is measured as diameter or radius in millimeter (zone of inhibition).

Drugs which exhibit large zones of inhibition are described as effective against the specified microorganisms, those which exhibit small or no zones of inhibition are described as ineffective against the microorganisms (contain little or no active ingredients), such drugs may also be described as counterfeit antimicrobials.

Therefore antimicrobial sensitivity tests can be microbiologically used to screen for counterfeit antimicrobial agent.

According to World Health Organization (WHO)

counterfeit medicines may include products with the correct ingredients but fake packaging, with the wrong ingredients, without active ingredients or with insufficient active ingredients.[1]

Counterfeit medicines represent an enormous public health challenge. Anyone, anywhere in the world, can come across medicines seemingly packaged in the right way, in the form of tablets or capsules that look right, but which do not contain the correct ingredients and, in the worst case scenario, may be filled with highly toxic substances. In some countries, this is a rare occurrence, in others, it is an everyday reality.[1]

Chemotherapeutic agents such as antibiotics, and antifungal agents are used for treating microbial diseases or infections.

The great demand of these medicines, poor regulatory authorities and corruption influence some pharmaceutical Industries to produce counterfeit antimicrobials.[2] Other factors such as poor quality control during manufacture and climatic conditions (poor storage) also contribute to the prevalence of counterfeit medicines in the pharmaceutical market.[1]

Patients unknowingly use some of the counterfeit medicines which illegally enter into the Pharmaceutical market. The consumption of such products leads to undesired and unpredictable effects to patients, drug resistance and even death.

Therefore the antimicrobial sensitivity study could screen for the presence of such counterfeits in the market and share such information with the Tanzania Food and Drugs Authority for further investigations so as to protect the public from the fatal effects of such pharmaceutical products.

Drug counterfeiting is reported to be a worldwide problem with the developing countries exhibiting higher prevalence rates.

About 1% of medicine sales in developed and up to 10% of medicine sales in developing countries are counterfeit.[1]

About one third of the WHO member states have poor means of controlling counterfeit medicines while up to 10% of drugs in developed and up to 25% of drugs in developing countries are counterfeit drugs.[2]

For instance, during a meningitis epidemic in Niger in 1995, more than 50,000 people were inoculated with fake vaccines resulting in 2500 deaths.[1]

In 2001, in South-East Asia, a Welcome Trust study revealed that 38% of 104 anti-malarial drugs on sale in pharmacies did not contain any active ingredients.[1]

In Cambodia, in 1999, at least 30 people died after taking counterfeit anti-malarials prepared with sulphadoxine-pyrimethamine (an older, less effective anti-malarial) which were sold as artesunate.[1]

According to a report released by the Organization for Economic Cooperation and development, 75% of counterfeits Worldwide come from India, some from Egypt and China.[3]

A recent report show that antibiotics, anti-malarials and antiviral agents are the most counterfeited medicines.[1]

It is also speculated that up to 40% of products labeled as containing artesunate (anti-malarial) contain no active ingredients and therefore have no therapeutic benefits. This is attributed to the counterfeiters' ability to reproduce holograms and other sophisticated printing techniques that had dramatically improved between 2001 and 2005, making detection even more difficult.[1]

The Food and Drugs Authority (FDA) estimates that up to 15% of all sold medicines in the World are counterfeits and in some parts of Asia and Africa figures exceed 50%.[4]

The United Nations humanitarian news reported that the busy Kariakoo market in the Tanzanian capital is stocked with knock-off merchandise - from imported car parts to handbags – and traders from across Africa come to buy cheap imports to sell at home. But the most dangerous counterfeits are the imitation medicines sold to unwitting consumers.[5]

METHODOLOGY

The disc diffusion method was used for the determination of antimicrobial activities.

MATERIALS

Apparatus used: Universal bottles, incubator, autoclave, Petri dishes, flasks, pipettes, Bunsen burner,

refrigerator, aluminium foil, and chemical balance.

Solvents used; Distilled water and dimethylsulfoxide (DMSO)

Media used were; Nutrient agar (NA) (Mumbai, India) and Sabouraud Dextrose agar (SDA) (Mumbai, India).

Test microorganisms; Standard strains of reference microorganisms;

Bacteria; *E. coli* and *Staphyloccocus aureus*

Fungi; *Candida albicans* and *Cryptoccocus neoformans.*

Antibiotics; Twenty samples of antibiotics (amoxicillin, ampicillin, dicloxacillin and ciprofloxacin).

Antifungal agents; Ten samples of antifungal agents (nystatin, ketoconazole and fluconazole).

PROCEDURES

Collection Of Samples

Twenty different types of antibiotics and ten samples of antifungals were randomly collected from different Pharmacies and Medical stores in Ilala Municipality.

The samples were weighed on electronic balance of which amoxicillin (250mg), ampicillin (250mg), dicloxacillin (250mg), ketoconazole (200mg), fluconazole (150mg) and nystatin (10mg) were separately suspended in 2.5ml of DMSO while 500mg of ciprofloxacin was suspended in 5.0ml of DMSO. About 20µl of each sample was impregnated on a 5mm diameter wide disc and left to dry before being deposited onto agar plates inoculated with strains of reference microorganisms against *E.coli* and *Staphyloccocus aureus* (bacteria) *and Candida albicans* and *Cryptoccocus neoformans (fungi)*. After an aerobic overnight incubation at 37ºC, mean zones of inhibition (ZI) were determined and recorded in millimeters.

Culture Media Preparation

About 14gm of NA and 32.5gm of SDA were respectively weighed and put into different conical flasks then 500ml of distilled water were added in each flask to form suspensions which were sterilized in an autoclave at 121ºC. The resulting hot solution was poured into Petri dishes and allowed to solidify at room temperature, after solidification the Petri dishes with the agar were refrigerated at about 4ºC for about 12 hours.

STOCK SOLUTION PREPARATION

About 250mg of each of the collected antimicrobial agents namely amoxicillin, ampicillin and dicloxacillin was dissolved in 2.5ml of DMSO, while 500mg of ciprofloxacin was dissolved in 5.0ml of DMSO.

Ketoconazole (200mg), fluconazole (150mg), and nystatin (10mg) were weighed and separately dissolved in 2.5ml of DMSO.

PREPARATION OF ANTIMICROBIAL DISCS

Several discs were punched out from a sheet of Whatmann® filter paper and each disc was impregnated with 20µl of the stock solution and then dried ready for use.

ANTIMICROBIAL ACTIVITIES TESTING

Two discs of each drug sample were tested for antibacterial activities against *E.coli* and *Staphyloccocus aureus*, while for antifungal activities *Candida albicans* and *Cryptoccocus neoformans* were employed as test microorganisms.

The inoculated Petri dishes with the test antimicrobial discs were incubated at 37ºC overnight and on the next day mean zones of inhibition were determined, recorded and interpreted accordingly.

RESULTS

Table 1: Analyzed samples of antimicrobial agents

Sample	Name	Source	Mean ZI (mm)			
			E. coli	*S. aureus*	*C.albicans*	*C.neoformans*
S1	Amoxicillin	Elys	21.0	21.0	ND	ND
S2	Amoxicillin	Zenufa	22.0	23.0	ND	ND
S3	Amoxicillin	Elys	21.0	23.0	ND	ND
S4	Amoxicillin	Zenufa	23.0	21.0	ND	ND
S5	Amoxicillin	India	22.0	21.0	ND	ND
S6	Ampicillin	India	22.0	20.0	ND	ND
S7	Ampicillin	Shelys	21.0	24.0	ND	ND
S8	Ampicillin	Keko	24.0	25.0	ND	ND
S9	Ampicillin	Shelys	23.0	22.0	ND	ND
S10	Ampicillin	India	24.0	23.0	ND	ND
S11	Ciprofloxacin	India	22.0	22.0	ND	ND
S12	Ciprofloxacin	India	23.0	24.0	ND	ND
S13	Ciprofloxacin	India	24.0	23.0	ND	ND
S14	Ciprofloxacin	India	22.0	24.0	ND	ND
S15	Ciprofloxacin	India	23.0	25.0	ND	ND
S16	Dicloxacillin	Keko	16.0	16.0	ND	ND
S17	Dicloxacillin	Shelys	15.0	18.0	ND	ND
S18	Dicloxacillin	Keko	13.0	15.0	ND	ND
S19	Dicloxacillin	Shelys	14.0	17.0	ND	ND
S20	Dicloxacillin	India	16.0	15.0	ND	ND
S21	Ketoconazole	Cyprus	ND	ND	18.5	19.0
S22	Ketoconazole	Microlabs	ND	ND	18.0	18.5
S23	Ketoconazole	Cyprus	ND	ND	16.5	17.5
S24	Nystatin	Cyprus	ND	ND	10.0	11.5
S25	Nystatin	Cyprus	ND	ND	12.0	12.0
S26	Nystatin	Cyprus	ND	ND	11.0	12.0
S27	Fluconazole	India	ND	ND	17.0	16.5
S28	Fluconazole	India	ND	ND	16.5	19.5
S29	Fluconazole	India	ND	ND	19.5	19.5
S30	Fluconazole	India	ND	ND	18.5	20.5

***ND**=not done.*

Table 2: Samples by respective sources/manufacturers

	Manufacturer							Total
Drugs	Elys	Zenufa	India	Shelys	Keko	Cyprus	Microlabs	
Amox	2	2	1	-	-	-	-	5
Amp	-	-	2	2	1	-	-	5
Cipro	-	-	5	-	-	-	-	5
Diclox	-	-	1	2	2	-	-	5
Ketaco	-	-	-	-	-	2	1	3
Nysta	-	-	-	-	-	3	-	3
Flucon	-	-	4	-	-	-	-	4
Total	**2**	**2**	**13**	**4**	**3**	**5**	**1**	**30**

Table3: Means of ZI yielded by test microorganisms against drug samples

Microbe	Drugs	Conc. (mg)	Manufacturer	Mean ZI (mm)
ST	AMOX	2.0	Elys	22.0
			Zenufa	22.0
			India	21.0
	AMP	2.0	India	21.5
			Shelys	23.0
			Keko	25.0
	CIPRO	2.0	India	23.6
	DIC	2.0	India	15.0
			Shelys	15.5
			Zeko	17.5
EC	AMOX	2.0	Elys	21.0
			Zenufa	22.0
			India	22.0
	AMP	2.0	India	23.0
			Shelys	22.0
			Keko	24.0
	CIPRO	2.0	India	22.8
	DICLO	2.0	India	16.0
			Shelys	14.0
			Keko	14.0
CA	KETOC	1.6	Cyprus	17.9
			Microlabs	17.0
	NYST	0,08	Cyprus	11.3
	FLUC	1.2	India	17.6
CN	KETOC	1.6	Cyprus	18.3
			Microlabs	17.5
	NYST	0.08	Cyprus	11.2
	FLUC	1.2	India	19.0

ST=*S.aureus, EC=E.coli, CA=C.albicans, CN=Cryptoccocus neoformans*

Table 4: Mean zones of inhibition regardless of the tested microorganisms

	Zone of inhibition (mm)		
Drugs	Mean	N	Std. Deviation
Amox	21.800	5	.9189
Amp	22.800	5	1.5492
Cipro	23.200	5	1.0328
Diclox	14.500	5	2.5495
Ketoco	17.583	3	1.0836
Nysta	11.250	3	.8660
Flucon	18.312	4	1.8154
Total	18.275	30	4.3282

Figure 1: ZI exhibited by various assayed drugs on test microbes

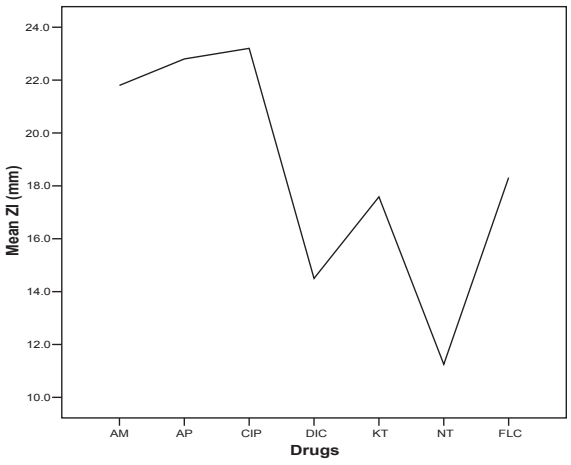
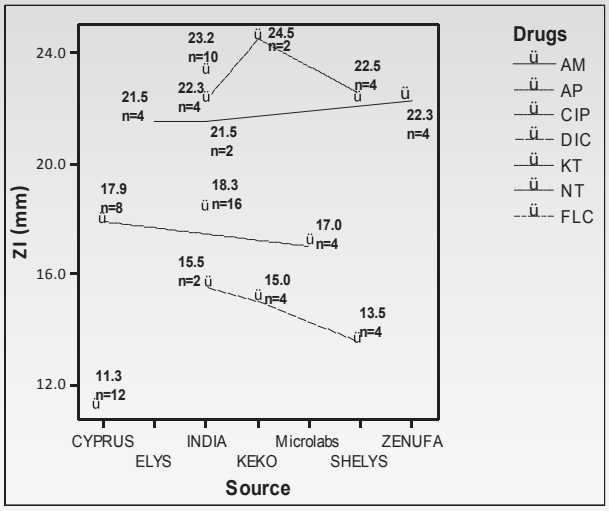


Figure2: Observed variability of results (ZI) of the tested drugs from different manufacturers



AM=amoxicillin, AP=ampicillin, CIP=ciprofloxacin, DIC=dicloxacilli n,KT=ketoconazole

NT=nystatin, FLC=fluconazole

DISCUSSION

A total of 30 samples of different antimicrobial agents were collected and microbiologically analyzed in this study. Of those 20 were antibacterial agents, namely amoxicillin, ampicillin and dicloxacilin capsules; each one with a total of 5 capsules. Antifungal agents were composed of ketaconazole, nystatin and fluconazole tablets (Table 1).

Variability in ZI was observed among the tested drugs. Though the differences in terms of millimeters were not statistically significant ($p<0.05$). However, this may have impact on the efficacy of the drugs against the microorganisms. For instance, ampicillin capsules from India, Shelly's and Keko differed among them by 3.5 mm. Ampicillin capsules manufactured in India being the least efficacious (Table 3)

For amoxicillin, ZI ranged between 21mm (Elys) and 23mm (Zenufa) against *E.coli.* Therefore a sample from Zenufa Laboratories was the most effective and a sample from Elys was the least effective, similarly a sample from Zenufa (23mm) was the most effective against *S.aureus* and a sample from Elys (21mm) was the least effective against *S.aureus (Table 1).* . For ciprofloxacin (India), the ZI ranged between 22mm and 25mm against both *E.coli* and *S.aureus* with the minimum effect (22mm) being against *E.coli* and the maximum effect (25mm) being against *S.aureus.* In case of dicloxacillin samples, a sample from Keko (13mm) was the least effective against *E.coli* and a sample from India (16mm) was the most effective against *E.coli, while ZI* between 15mm (India) and 18mm (Shelys) were exhibited by ampicillin samples against *S.aureus (Table 1)*

On the other hand ketoconazole exhibited ZI ranging between 16.5mm and 19.0mm against both *Candida albicans* and *Cryptoccocus neoformans* with the maximum effect being against *Cryptoccocus neoformans* and minimum effect being against *C.albicans.* Nystatin exhibited ZI ranging between 10mm and 12mm against both fungi. For the case of fluconazole, from 16.5mm and 20.5mm were exhibited against both fungi with the maximum effectiveness being against *C.neoformans (Table 1)*

Generally speaking, regardless of the tested microorganisms, ciprofloxacin exhibited the largest mean radius (zone of inhibition) (23.3mm) followed by Ampicillin (22.8mm) as indicated on Table 4 and figure 1. Nystatin samples exhibited the least mean zone of inhibition.

The slight variations in effectiveness observed among the same class of antimicrobial agents (for example amoxicillin samples) may be due to differences in formula used by different manufacturers or inter batch variations or slight differences in active ingredients.

Figure 2 show the observed variability of results (ZI) of the tested drugs from different manufacturers. For instance, dicloxacillin from India, Keko and Sheyls varied from as low as 13.5 to 15.5 mm; as well as ampicillin from Ellys, Zenufa and India varied from 21.5- 22.3mm, suggesting the existence of slight variations in efficacy among drugs from different manufacturers.

One of the drawbacks of this study was the lack of pure standard powder of each antimicrobial agent for preparing discs for comparison with the effects of the sample discs.

CONCLUSSION AND RECOMMENDATION

The antimicrobial agents analyzed in this study have demonstrated substantial antimicrobial (antibacterial and antifungal) activities against the test microbes; an indicative of possession of active ingredients.

As far as the *in vitro* microbiological assays are concerned, the study's findings could not reveal any counterfeit drug.

However, further studies should be conducted to confirm the content specifications and other relevant parameters of each pharmaceutical preparation, because of lack of uniformity among the results in terms of ZI within the same class of drugs from different manufacturers.

REFERENCES

1. *World Health Organization: counterfeit and substandard medicines: Fact sheet revise 2006.*
2. ***J****ournal of Antimicrobial Chemotherapy-counterfeit or substandard antimicrobial drugs: A review of scientific evidence, 2007.*
3. *Wikipedia, the free encyclopedia, searched on 10th December, 2008 at 10:30*
4. *World Health Organization-counterfeit and substandard medicines. 2004.*
5. *World Health Organization-counterfeit and substandard medicines. 2004.*