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ORIGINAL RESEARCH

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Contribution of storage conditions of antibiotics in pharmacies on efficacy loss of amoxicillin and tetracycline against strains of *Escherichia coli* and *Staphylococcus aureus* in the city of Lome

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ABSTRACT

Background: The resistance of bacteria to antibiotics is now a public health issue. The causes of such resistance are multiple and some are related to non-compliance with recommended doses of antibiotics. The storage conditions of antibiotics can have an impact on their effectiveness. Indeed, at a temperature above 30°C and relative humidity above 60%, pharmaceutical presentations of antibiotics may lose their doses. The goal of our study was to evaluate the existence of a correlation between failures of antibiotic therapy and poor conservation of marketed antibiotics in the city of Lome.

Methods: We had prospected five pharmacies in the city of Lome and have collected in each, four pharmaceutical presentations of antibiotics. These antibiotics are amoxicillin, ciprofloxacin, tetracycline, and chloramphenicol. Also, we isolated in two private laboratories in the city of Lome, five hospital strains of these following bacteria: *Escherichia coli, E. coli* Alkalescens-Dispar, *Klebsiella pneumonia*, and two strains of *Staphylococcus aureus*. Reference strains of *K. pneumoniae* ATCC 13883, E. coli ATCC 25922, S. aureus ATCC 29213, *S. aureus* ATCC 25923, and Pseudomonas aeruginosa ATCC 27853 were used for analysis control. We carried out a prospective investigation and an analysis of the effectiveness of the antibiotics by the method of diffusion in agar medium.

Results: The survey results showed that all pharmacies surveyed had an ambient temperature above what is recommended. *In vitro* analysis of the efficacy of amoxicillin on hospital strains of *E. coli* revealed some resistances. Also, the *in vitro* analysis of the tetracycline on reference strains of *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 was found to be ineffective.

Conclusion: The storage conditions of these antibiotics, especially the temperature of their conservation, could be responsible for the loss of their effectiveness. It is possible that, in the city of Lome, some resistances of bacteria to marketed antibiotics are due to inappropriate conservation conditions of these products.

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Introduction

Antibiotics are one of the most important discoveries of our millennium; they revolutionized therapeutics by being effective solutions for previously incurable diseases. Unfortunately, this therapeutic revolution is in danger. In fact, the bacteria that were previously sensitive to the action of these antibiotics develop mechanisms of resistance to them [1–3].

These resistances are explained by the appearance of random genetic mutations of the bacteria or exchanges of genes of resistance between the bacteria [4]. Also, the misuse or irregularities during treatment or unspecified doses of antibiotics contribute to this appearance of resistance [5]. The consequences are disastrous: it goes away from the therapeutic failures to the aggravation of the disease. And if nothing

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is done, today's antibiotics will become ineffective tomorrow on the same diseases [6,7].

In pharmaceutical, oral marketed antibiotics should be stored at an ambient temperature below 25°C and relative humidity below 60%, unless there is another specification given by the manufacturer [8]. At higher temperatures, the ingredients may decompose and the drug may become ineffective or even toxic and even microbiologically contaminated [9].

The following work evaluated the existence of a correlation between failures of antibiotic therapy and poor conservation of marketed antibiotics in the city of Lome. More specifically, as a first step, we established the storage conditions of marketed antibiotics in the city of Lome through measurements and data collected in pharmacies, then we analyzed the resistance of bacterial strains to these antibiotics, to whom they are normally sensitive.

Material and Methods

Materials

Investigation sheets

They provide information on the storage conditions of antibiotics in five pharmacies of the city of Lome, especially ambient temperature and relative humidity.

Thermo-hygrometer

The ambient temperature and relative humidity were measured using the KTJ TM Thermo-hygrometer.

Biological material

Five hospital bacterial strains isolated in two private laboratories in the city of Lome of these following bacteria: *E. coli*, *E. coli* Alkalescens-Dispar*, K. pneumonia*, and two strains of *Staphylococcus aureus*.

Reference strains of *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used for analysis control. Table 1 shows the Recapitulate of Reference strains used.

Culture center

Tryptone Sel broth (BIO-RAD, France) was used to prepare bacterial suspensions. Antimicrobial assays were performed with Mueller-Hinton medium (Oxoid, UK) supplemented with the antibiotic to be tested.

Used antibiotics

We used antibiotic disks of amoxicillin, ciprofloxacin, chloramphenicol, and tetracycline (BIO-RAD, France) to determine the sensitivity of hospital strains and marketed antibiotics collected in pharmacies (amoxicillin, ciprofloxacin, chloramphenicol, and tetracycline) to study the impact of temperature

Amoxicillin

It belongs to the class of beta-lactams, specifically aminopenicillins or penicillins A. It is characterized by a broad spectrum of action affecting Gram-positive and Gram-negative bacteria that do not produce penicillinases. It is administered orally because of its acid resistance [10]. The basic nucleus is the beta-lactam ring. It is bactericidal and causes blocking of the enzymatic activity of proteins by fixing penicillins, leading to the cessation of the synthesis of peptidoglycan which is the main component of the bacterial wall [3].

Ciprofloxacin

It is a fluoro-quinolone, a synthetic antibiotic. It has a bactericidal action by inhibition of bacterial DNA gyrase resulting in the blocking of protein synthesis [3].

Tetracycline

It has a bacteriostatic action by inhibiting protein synthesis through binding to the 30S subunit of the bacterial ribosome [3]. They are indicated to fight

Table1. Recapitulate of reference strains used.

Bacteria strain	Antibiogram	
	Sensitive	Resistant
E. coli ATCC 25922	Amoxicillin, Ciprofloxacin, Chloramphenicol, Tetracycline	
K. pneumoniae ATCC 13883	Ciprofloxacin	Amoxicillin, Chloramphenicol, Tetracycline
S. aureus ATCC 29213	Amoxicillin, Ciprofloxacin Chloramphenicol, Tetracycline	
S. aureus ATCC 25923	Ciprofloxacin, Chloramphenicol, Tetracycline	Amoxicillin
P. aeruginosa ATCC 27853	Ciprofloxacin	Amoxicillin, Chloramphenicol, Tetracycline

against Gram-positive, Gram-negative, and anaerobic bacterial infections, but also against certain other microorganisms (Chlamydia, Mycoplasma).

Chloramphenicol

It is a part of the family of Phenicolates and has a bacteriostatic action that limits the synthesis of proteins by inhibiting peptidyl transferase activity and bacterial ribosome. These are small, hydrophobic molecules that easily cross the outer and inner membrane of Gram-negative bacteria [3].

Data on marketed antibiotics

The chosen antibiotics are among the best-selling antibiotics in the city of Lome and their characteristics are shown in Table 2.

Methods

Isolation and identification of hospital bacterial strains

Cytological examination was performed as well as Gram staining. Chapman, Bromo Cresol Pourpre, Drigalski, and Eosin Methylene Blue agar media were seeded. Pus was sown on the chocolate blood agar. The urine was directly seeded on the Uriselect medium. After 24 hours incubation at 37°C aerobically, the different types of colonies present were subjected to control Gram staining and the identification was made by conventional biochemical tests (catalase, oxidase search), nitrate reductase, coagulase, urease, tryptophan deaminase, indole, and the use of glucose and mannitol).

Sensitivity profile of hospital bacterial strains isolated

The sensitivity profile was carried out by the disk diffusion method according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [11]. Table 3 recapitulates the hospital strains used.

Evaluation of the effectiveness of antibiotics collected at the pharmacy

For antimicrobial testing with pharmaceutical antibiotic presentations, the Kirby-Bauer method was modified and the modification involved the direct incorporation of the antibiotic into the agar at the time of agar preparation [12]. Different dilutions of antibiotics were added at specific volumes of the liquid agar of Muller-Hinton, before its distribution in Petri dishes. We obtain culture media at different concentrations of antibiotics: 2.5, 5, and 1 µg/ml. Table 4 summarizes the composition of the reaction media. The bacterial strain is considered antibiotic-sensitive when there is no bacterial growth and is considered resistant when bacterial growth is observed.

Results

Ambient temperature of pharmacies

The average ambient temperature of pharmacies was 29.9°C with extremes ranging from 26.3°C to 32.5°C. All pharmacies had an ambient temperature that exceeded 25°C, as well as the findings of Suhail et al. [13]. Two of these pharmacies surveyed had

Table 2. Data on marketed antibiotics.

Trade name	DCI	Antibiotic class	Pharmaceutical company	Storage conditions indicated by the manufacturer	Expiration date
Clamoxyl 500 mg	Amoxicillin	Beta-lactam	GlaxoSmithKline	Do not store above 25°C in the outer carton in order to protect from moisture.	01/2019
Ciplox 500 mg	Ciprofloxacin	Quinolone	Cipla	Do not store above 30°C	12/2018
Tetracycline 250 mg	Tetracyclin	Tetracyclin	GGIA	No manufacturer information	03/2019
Chloramphenicol 250 mg	Chloramphenicol	Phenicol	GGIA	No manufacturer information	03/2019

DCI: International common name.

Table3. Recapitulate of hospital strains used.

Sampling type	Identified Bacteria strain	Sensitivity	Resistance
Urines	*E. coli*	Amoxicillin, Ciprofloxacin, Tetracycline	Chloramphenicol
Urines	*E. coli* Alkalescens-Dispar	Ciprofloxacin	Amoxicillin, Tetracycline Chloramphenicol,
Urines	*K. pneumoniae*	Ciprofloxacin, Choramphenicol	Amoxicillin, Tetracycline
Urines	*S. aureus*	Amoxicillin, Ciprofloxacin, Choramphenicol	Tetracycline
Pus	*S. aureus*	Amoxicillin, Ciprofloxacin, Choramphenicol	Tetracycline

Table 4. Composition of the reaction media.

Concentration obtained (µg/ml)	10	5	2,5
Amount of antibiotic (µg)	1,000	500	250
Quantity of Mueller Hinton agar (ml)	100	100	100

a temperature above 30.0°C, conservation temperature at which antibiotics can degrade [9].

Relative humidity rate

The average relative humidity obtained is 49.3%, with extrema between 46.5% and 51.5%. In total, no pharmacy had a relative humidity higher than the 60% recommended [8].

Air cooling system

Only one of the pharmacies surveyed had a functional air conditioner at the time of the survey. The temperature in that pharmacy was 26.3°C. Two pharmacies used fans and their temperature was just fairly below 30°C. The recapitulate of pharmacies surveyed is in Table 5.

Antibiotic susceptixbility profile of bacterial strains

The antibiogram results are shown in Table 6. The analysis of the results reveals two particularities. The strain of *E. coli* isolated from urine was found resistant to amoxicillin (2.5 µg/ml) taken from all pharmacies except amoxicillin from pharmacy PB which inhibited the growth of this strain; in addition, at a dose of 5 µg/ml, this strain still resisted amoxicillin from a pharmacy PD. The results are shown schematically in Figure 1. At the dose of 5 µg/ml, the reference strains of *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *S. aureus* ATCC 25923 were found resistant to tetracycline from the pharmacy PB.

The *in-vitro* analysis with marketed antibiotics of ciprofloxacin and chloramphenicol was consistent with pre-established antibiogram results of bacterial strains.

Discussion

The bacterial strain *E. coli* isolated from urines was found resistant to amoxicillin (2.5 µg/ml) from all pharmacies, except that from pharmacy PB that inhibited the growth of this strain. Also, at the dose of 5 µg/ml, this strain still resisted amoxicillin from pharmacy PD. The pharmacy PB has recorded the lowest temperatures (average ambient temperature 26.3°C), whereas the pharmacy PD is among those with the highest product storage temperatures (average ambient temperature of 29.6°C). According to the recommendations of

Table 5. Recapitulate of pharmacies surveyed.

Code of investigated Pharmacies	Average ambient temperature (°C)	Air cooling system	Relative humidity rate (%)
PA	32.5	None	50.5
PB	26.3	Air conditioner	46.5
PC	29.1	Fan	48.0
PD	29.6	Fan	50.0
PE	31.7	None	51.5

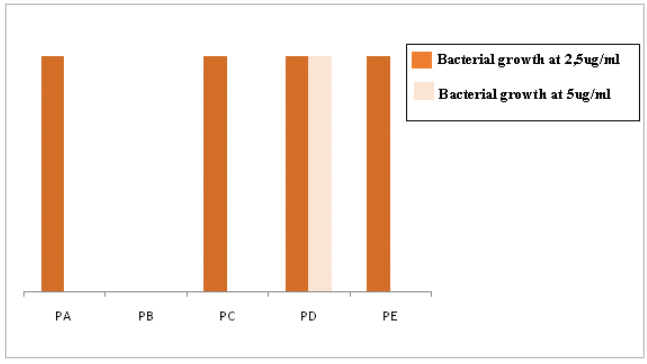

Figure 1. Action of amoxicillin on the strain of *E. coli* isolated from urines.

GlaxoSmithKline laboratory, manufacturer of this pharmaceutical presentation of amoxicillin, the storage temperature must not exceed 25°C. This may be a factor explaining the inefficacy of amoxicillin on the *E. coli* strain tested. The decrease in the efficacy of the pharmaceutical presentations of amoxicillin under the effect of temperature would be caused by the destruction of the lactam nucleus [9].

Tests conducted with amoxicillin from the majority of pharmacies were unsatisfactory; some pharmacies provided poor quality amoxicillin [14]. Studies in South Africa have estimated a loss of efficacy of amoxicillin tablets at temperatures around 30°C [15]; indeed, amoxicillin loses its effectiveness in inadequate storage conditions [16–18].

At a dose of 2.5 µg/ml, the reference strains of *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *S. aureus* ATCC 25923 were found resistant to tetracycline from the pharmacy PB. Although the average ambient temperature recorded in the pharmacy PB did not exceed the temperature of 30°C, it did not comply with the general recommendations. Indeed, as well as Crichton's study, any drug storage temperature, whether lower or higher than 25°C in a pharmacy impacts the efficacy of the drug [19].

However, other factors besides storage conditions may influence the resistance of bacterial strains to

Table 6. Sensitivity profile of antibiotic strains marketed in pharmacies.

	PA						PB								PC								PD								PE					
bacteria	AMX		CIP		C		TE		AMX		CIP		C		TE		AMX		CIP		C		TE		AMX		CIP		C		TE		AMX		CIP		C		TE	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Ec1	R	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	R	S	S	S	ND	ND	S	S	R	R	S	S	ND	ND	S	S	R	R	S	S	ND	ND	S	S
Ec2	S	S	S	S	ND	ND	ND	ND	S	S	S	S	ND	ND	ND	ND	S	S	S	S	ND	ND	ND	ND	S	S	S	S	ND	ND	ND	ND	S	S	S	S	ND	ND	ND	ND
Ec3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Kp1	ND	ND	S	S	S	S	ND	ND	ND	ND	S	S	S	S	ND	ND	ND	ND	S	S	S	S	ND	ND	ND	ND	S	S	S	S	ND	ND	ND	ND	S	S	S	S	ND	ND
Kp2	ND	ND	S	S	ND	ND	ND	ND	ND	ND	S	S	ND	ND	ND	ND	ND	ND	S	S	ND	ND	ND	ND	ND	ND	S	S	ND	ND	ND	ND	ND	ND	S	S	ND	ND	ND	ND
Sa1	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND
Sa2	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND
Sa3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Sa4	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	R	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S	ND	ND	S	S	S	S	S	S
Pa	ND	ND	S	S	ND	ND	ND	ND	ND	ND	S	S	ND	ND	ND	ND	ND	ND	S	S	ND	ND	ND	ND	ND	ND	S	S	ND	ND	ND	ND	ND	ND	S	S	ND	ND	ND	ND

AMX = amoxicillin; CIP = ciprofloxacin; C = chloramphenicol; TE = tetracycline.
R = resistant; S = sensitive; ND = not determined.
1: Muller-Hinton Agar with an antibiotic concentration of 2.5 µg/ml.
2: Muller-Hinton Agar with an antibiotic concentration of 5 µg/ml.
Ec1: *E. coli* isolated from urines.
Ec2: *E. coli* Alkalescens-Dispar isolated from urines.
Ec3: *E. coli* ATCC 25922.
Kp1: *K. pneumoniae* isolated from urines.
Kp2: *K. pneumoniae* ATCC 13883.
Sa1:*S. aureus* isolated from urines.
Sa2: *S. aureus* isolated from pus.
Sa3: *S. aureus* ATCC 29213.
Sa4: *S. aureus* ATCC 25923.
Pa: *P. aeruginosa* ATCC 27853.

this antibiotic. In this study, the marketed antibiotics used for every type of antibiotic perish at the same date; the life of these presentations is, therefore, the same and cannot be involved to explain differences. However, the length of stay in pharmacy has not been evaluated as well as factors related to the routing of the manufacturer to the pharmacy.

In the city of Lome, some pharmacies do not offer ideal storage conditions for the pharmaceutical products sold, like a temperature higher than the recommended. Thus, inadequate storage conditions for pharmaceuticals would probably have contributed to the decrease in the efficacy of amoxicillin on bacterial strains. The problem of the marketed antibiotic inefficacy deserves to be taken seriously and, studies on a larger scale must be carried out taking into account of physicochemical parameters such as exposure to light and radiation, the length of stay in pharmacy, the process of transporting the products from the manufacturer to the pharmacy, and the remaining life of the molecule.

Conclusion

In the city of Lome, some pharmacies do not offer ideal storage conditions for the pharmaceutical products sold, like a temperature higher than the recommended. Thus, inadequate storage conditions for pharmaceuticals would probably have contributed to the decrease in the efficacy of amoxicillin on bacterial strains. The problem of the marketed antibiotic inefficacy deserves to be taken seriously and, studies on a larger scale must be carried out taking into account of physicochemical parameters such as exposure to light and radiation, the length of stay in pharmacy, the process of transporting the products from the manufacturer to the pharmacy, and the remaining life of the molecule.

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