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Article

Urinary bacterial resistance to nitrofurantoin in Maputo, Mozambique

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Abstract: Urinary tract infections are a major cause of morbidity and mortality in Mozambique. They are sometimes treated empirically with nitrofurantoin. However, little is known about this antibiotic's performance and bacterial resistance in the country. This study analyzed the results of nitrofurantoin sensitivity tests requested in the Central Hospital of Maputo during 2012 and 2013. As result, 181 samples were tested and most cases (66.9%) showed absolute sensitivity but there were considerable cases of resistance (29.8%). *Morganella morganii* was the only bacteria presenting no absolute or intermediate resistance. The sensitivity was also high in the case of *Escherichia coli* (90%) and Gram-negative bacteria (66.7%). *Serratia marcescens* was mostly resistant (64.3%). The remaining bacteria showed inconclusive results. Thus they shall be subjected to a sensitivity test before prescription. Factors such as seasonality, patients' sex and urine transparency did not seem to be reliable indicators of microbial resistance in the urine. Yet, a longer time span (over 5 years) might be sufficient for the sensitivity profile to change.

Keywords: urine, resistance, antibiotic, nitrofurantoin, Mozambique

1. Introduction

Nitrofurantoin is a major bladder antibiotic, included in the WHO Model List of Essential Medicines [1]. It efficacy has been shown in Mozambique [2,3] but it is rarely used because of its limited availability, broad spectrum of activity, restricted for uncomplicated urinary infections or alleged side effects. However, it might be wise to reconsider its more frequent use because the most common antibiotics are becoming less effective.

The country recently joined the Global Antibiotic Resistance Partnership (GARP) and ran an assessment of the current status, but found very little information [4]. Yet, there is some evidence of antimicrobial drug resistance in Mozambique. Some authors observed the phenomenon in a rural hospital from the southern region [5,6], and Apalata, *et al.* [7] detected it in *Neisseria gonorrhoeae* collected from patients from the Central Hospital of Maputo. This is concerning considering disorders such as urinary tract infections, described by Tessema, *et al.* [8] as a significant causes of morbidity and mortality.

Among the few studies in the country, only two original included nitrofurantoin antimicrobial tests: Ceccarelli, *et al.* [2] studied the resistance of *Staphylococcus aureus* to several antibiotics, using pus samples from abscesses; van der Meeren, *et al.* [3] focused their studies on urine pathogens in hospitalized children from Beira. The former study is only about *S. aureus*, not considering several other pathogens. The latter was the only one where nitrofurantoin was tested for urine samples. Yet, they did not broaden their study to the general population and the children were already under a

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multi-antibiotic treatment. In such cases, the microbial flora had already been conditioned to some extent.

Thus, this study aimed to profile the bacterial sensitivity to nitrofurantoin using urine samples from male and female patients disregarding their age or other socio-demographic factors. It intends to provide a more concrete idea about the range of bacteria affected and how resistant they are to the antibiotic.

2. Results

From 276 urine samples, 181 were effectively analyzed for nitrofurantoin resistance (Table 1). It consisted urine from 120 female subjects and 61 male. It was a significant disparity ($p < 0.001$). Differences also took place in the frequency of cases throughout the year ($p = 0.005$), with the highest number in April and May (24 subjects) and lowest in June (6). Yet, almost the same number of cases was observed in rainy (90) and dry (91) seasons. According to this observation, average female patients treated with nitrofurantoin are twice as more if compared to male, varying in number over the months.

Table 1. Frequency of cases tested for nitrofurantoin.

Month	Frequency of cases		Total
	Female	Male	
January	21	2	23
February	8	2	10
March	14	5	19
April	19	5	24
May	12	12	24
June	5	1	6
July	6	5	11
August	3	9	12
September	9	5	14
October	8	6	14
November	9	5	14
December	6	4	10
Total	120	61	181

p-value = 0.014

Nitrofurantoin was effective in most cases (**Table 2**) but almost one third of the samples showed mild to complete resistance. The findings indicate that nitrofurantoin is often effective or not, being rarely in the "gray area".

Table 2. Overall resistance to nitrofuratoin.

Resistance	Frequency	Percent (%)
Sensitive	121	66.9
Intermediate	6	3.3
Resistant	54	29.8
Total	181	100.0

p-value < 0.001

There were 15 different genera or groups of pathogens but only 6 present in more than 5% of the samples (Figure 1). A Kolmogorov-Smirnov test shows significant quantitative differences considering the species. *Escherichia coli* was the most frequent, followed by *Klebsiella pneumoniae* and Gram-negative bacteria.

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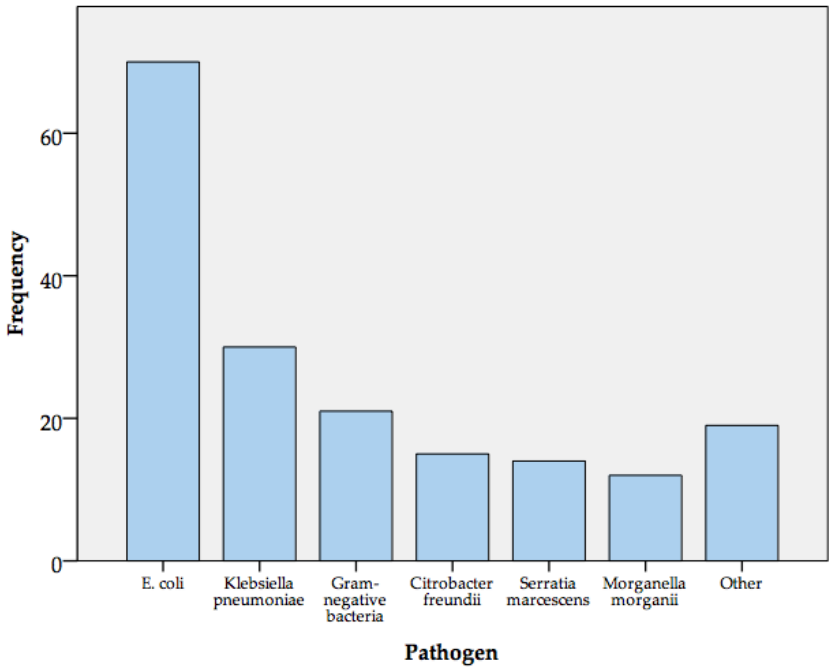

Figure 1. The major pathogens found in the urine samples.

Proteus mirabillis and other minor pathogens were present in 19 samples, together making approximately 10%. This implies a certain probability to find more frequently some pathogens in relation to others among people carrying urinary tract diseases.

Pathogens exhibited different sensitivity profiles (**Table 3**). *S. marcenscens* showed the highest resistance (64%) and *M. morganii* was the most sensitive, not exhibiting any case of absolute or mild resistance. However, *S. marcenscens* also exhibited the highest percentage of intermediate resistance, and this makes the microorganism less predictable than the others. E. coli and Gram-negative bacteria were mostly sensitive but the remaining showed inconclusive results. Thus, the antibiotic is more active in some pathogens in relation to others.

Table 3. Antibiotic sensitivity profile of the microbes detected. Note: there are more pathogens but these were the ones with representative frequencies.

Pathogen	Frequency	Resistance (%)			
		Sensitive	Intermediate	Resistant	Total
Serratia marcescens	14	21.4	14.3	64.3	100
Citrobacter freundii	15	46.7	0	53.3	100
K. pneumoniae	30	43.3	3.3	53.3	100
Proteus mirabillis	8	50	0	50	100
Gram-negative bacteria	21	66.7	4.8	28.6	100
E. coli	70	90	2.9	7.1	100
Morganella morganii	12	100	0	0	100
Overall	170	59.7	3.6	36.7	100

p-value < 0.001

Resistance was also analyzed throughout the year assuming that people change behaviors such as diet, physical activity, resting or urinating patterns according to the seasons. It is reasonable to assume such factors can affect the urinary tract's microbial flora. Yet, the sensitivity did not seem to change significantly at $\alpha = 0.05$ as p-value was 0.413 (Table 4). Indeed, the sub-average resistances

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showed similar profiles for both seasons. These results suggest that urine pathogens do not present variation in nitrofurantoin resistance during a year-round.

Table 4. Average annual sensitivity profile for nitrofurantoin from 2012 and 2013.

Season	Month	Resistance (%)			
		Sensitive	Intermediate	Resistant	Total
Dry	April	45.8	12.5	41.7	100
	May	62.5	0	37.5	100
	June	66.7	0	33.3	100
	July	81.8	0	18.2	100
	August	83.3	0	16.7	100
	September	71.4	0	28.6	100
	Sub-average	68.6	2.1	29.3	100
Rainy	October	71.4	0	28.6	100
	November	71.4	7.1	21.4	100
	December	90	0	10	100
	January	60.9	4.3	34.8	100
	February	80	10	10	100
	March	57.9	0	42.1	100
	Sub-average	71.9	3.6	24.5	100
Average		70.3	2.8	26.9	100

p-value = 0.413

The pathogens isolated from patients of opposite sexes showed visibly dissimilar sensitivity profiles (Table 5), although the difference was not significant for $\alpha = 0.05$ (p-value = 0.069). The resistance percentage was consistently lower than sensitivity. The samples isolated from female subjects were relatively more sensitive than the ones from male.

Table 5. Sensitivity profiles considering the sex.

Resistance	Percentage (%)		
	Female	Male	Average
Sensitive	71.7	57.4	66.9
Intermediate	1.7	6.6	3.3
Resistant	26.7	36.1	29.8
Total	100	100	100

p-value = 0.069

Finally, urine transparency was also analyzed to see if it shows some relationship to the sensitivity to nitrofurantoin. However, it was necessary to determine whether or not the transparency reflected the presence of pathogens. A binomial test showed significant differences between the quantity of clear (65) and turbid (116) urine (p-value < 0.001). However, different microorganisms did not seem directly related to a particular state of transparency (Table 6). The difference was not significant for $\alpha = 0.05$ and the results cannot be conclusive. Thus, the urine turbidity is not likely to be associated with the microbial resistance to nitrofurantoin. The highest percentage of sensitive bacteria in turbid urine might be simply because of the higher contamination in these samples.

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Table 6. Sensitivity profiles considering the transparency.

Resistance	Percentage (%)		
	Clear	Turbid	Total
Sensitive	61.5	69.8	66.9
Intermediate	1.5	4.3	3.3
Resistant	36.9	25.9	29.8
Total	100	100	100

p-value = 0.212

3. Discussion

There is limited literature on this particular matter from Mozambique to discuss with. The most similar studies have narrower scope. The study by van der Meeren, *et al.* [3] only considered children. However, it had a similar methodology and was also held in Mozambique, though in a different hospital. Rodrigues and Barroso [9] said the microbial flora of urinary tract infection patients vary with age, and there is a distinction between people below and above 16 years old. The current was designed to randomly pick a broader group. Furthermore, the study in children registered several cases of HIV-positive samples, and it certainly affected their immunity and their treatments. Ceccarelli, *et al.* [2] were only concerned about *S. aureus*, , neglecting the wide variety of urinary tract pathogens. Thus, this study is more representative of what is happening in Mozambique, especially in Maputo City.

The highest frequency of female patients is certainly in part because it represents country's population profile, with more women than men, and also women are more vulnerable to urinary tract infections [10]. Rodrigues and Barroso [9] also observed a similar demographic profile in Portugal. According to them, the higher feminine susceptibility is due to the ureter's proximity to the anus, making it easy for fecal bacteria to invade. Yet, this demographic feature is not probably a problem for this research because both male and female subsets were in frequencies high enough for a reliable statistical treatment in the SPSS software ($n > 8$). Also, it was possible to obtain an acceptable number of patients in most months to visualize what happens throughout an entire year.

The abundance of *E. coli* and *K. pneumoniae* is common in urine pathogen studies [3,11], even in other countries or contexts such as Portugal [9], Brazil [12,13] and India [14]. These microorganisms are able to adhere to the cells and effectively resist to the urine flow [15]. *E. coli* is highly abundant in the intestines as part of the normal flora. Its adhesins and exotoxins facilitate the colonization of the urethra and then the bladder [16,17]. Indeed, profiles just like the one found in this study have been frequently found [9,18], and it suggests there is a common profile in the current study.

Morganella morganii was the only bacteria absolutely sensitive to nitrofurantoin. All the others showed some degree of resistance. *E. coli* was also highly sensitive (90%), and the unidentified Gram-negative bacteria were mostly sensitive (66.7%). The remaining showed equal degree of resistance and sensitivity (*Proteus mirabillis*) or were resistant in most cases. *E. coli*'s resistance (7.1%) was close to the level found in the hospitalized in the city of Beira (9.1%) [3]. *K. pneumoniae* showed more cases of resistance (53.3%) than sensitivity (43.3%), but Mshana, *et al.* [11] calculated only 20% of resistance in Tanzania. These differences could have been geographic but one cannot discard the possibility of a different dosage or if there were complementary medications for the Tanzanian studies. The authors did not specify such details in their meta-review and they say that some articles did not explain which guidelines they used to analyze bacterial susceptibility to antibiotics. Furthermore, susceptibility changes over time [9] and it makes difficult to compare studies performed at different periods.

Most cases showed sensitivity to nitrofurantoin and it is desirable for a medicine. Indeed, experiments by van der Meeren, *et al.* [3] and Ceccarelli, *et al.* [2] showed little resistance to nitrofurantoin, independently of the microorganisms. For van der Meeren, *et al.* [3], there was 9.1% of resistance for *E. coli* and 11.1-25% of resistance for *K. pneumoniae*, and in case of Rodrigues and Barroso [9], *E. coli* and *K. pneumoniae* showed no resistance to nitrofurantoin. Sinha and Benny [14]

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found sensitivity in 73.4% of their samples from a tertiary care teaching hospital. Actually, nitrofurantoin appears to have a wide array of target microbes. Indeed, Huttner, *et al.* [19] recommended doctors to prescribe it in the short term for simple cases of urinary tract infections. But in this study, 29.8% of the cases showed resistance and 3.3% intermediate resistance. Mshana, *et al.* [11] mentioned an even higher level of resistance (37%) in their meta-study from urine studies in Tanzania. The resistance found in the current study and the Tanzanian are probably more representative of the general populations' profiles because they covered a wider range of microorganisms found and their distinct resistances to the antibiotic. They were collected from subjects with different ages, and adults shall be expected to have a broader array of urinary tract microbial flora due to habits such as the sexual activity, exchanging body fluids. Thus, the current study's results are very plausible but the context matters. In Mozambique, nitrofurantoin still seems a good empiric option to treat *E. coli*, *M. morganii* and possibly Gram-negative bacteria but not for the remaining pathogens.

According to the current results, there is no significant variation in sensitivity throughout the year. Two years might not be enough to see significant changes, but Rodrigues and Barroso [9] reported changes in efficacy of several antibiotics, in Portugal. However, their study showed no resistance to nitrofurantoin and it has not changed from 2002 to 2007. However, the articles by van der Meeren, *et al.* [3] and Mshana, *et al.* [11] were performed in 2013 in Mozambique and Tanzania, respectively.

Concerning the sex, the results were inconclusive (p-value = 0.069) but the bacteria isolated from male subjects seemed more resistant at first glance. Sinha and Benny [14] said antimicrobial resistance is in fact more likely to be found in urine samples from male patients. Sahuquillo-Arce, *et al.* [20] reached the same conclusion in their retrospective analysis of data from 16 hospitals in Spain.

Finally, urine transparency seems to say little on the bacterial sensitivity or resistance to nitrofurantoin. The difference between clear and turbid urine was not significant (p-value = 0.212) and the Mozambican literature available does not focus much on this variable. The transparency probably says more about the quality and even state of infection of the urine, but not necessarily about the microbial resistance.

4. Materials and Methods

4.1. Ethical considerations

The study abided the 2008 revision of the Declaration of Helsinki. The patients' confidentiality had be assured through the omission and codification of their names even before the data collection, the data do not present their addresses, it was not invasive or interventional, the primary data before the statistic treatment was not shared with anyone besides the researchers involved, and the study did not discriminated races, religions, ethnicities, political preferences, interest groups and did not offend any particular entity. The gender was simply used as a variable for an academic purpose. The study was carried out in accordance with the Declaration of Helsinki, and the Institutional Bioethics Committee of the Faculty of Medicine and Central Hospital of Maputo approved the protocol in July 2014 under the code CIBS FM&HCM/57/2014. The consent form does not seem necessary because the authors did not have access to the patients' identities and the information collected was not detailed enough to allow the patients to be tracked.

4.2. Procedure

The study was transversal and retrospective. The data were retrieved from the Laboratory of Microbiology of the Central Hospital of Maputo and it consisted of 276 results of antibiotic sensitivity tests from urine samples requested from January 2012 to December 2013. The cultures involved were positive to bacteria (counts > 105 CFU/mL) and excluded patients with negative results and without antibiotic sensitivity tests. The variables studied were the year, month, the subjects' sex, pathogen and the sensitivity to nitrofurantoin.

Urine transparency had been analyzed according as described by Strasinger and Lorenzo [21]. They divided the samples in three categories: clear, slightly turbid and turbid. The following urine cultures had been carried out according to the Brazilian National Agency for Health Surveillance guidelines [22]. They were conducted in cystine lactose electrolyte deficient agar (CLED) to allow the growth of pathogens and then MacConkey differential medium to select the Gram-negative bacteria. The nitrofurantoin sensitivity tests had been conducted according to the guidelines published by the National Committee for Clinical Laboratory Standards (NCCLS) [23].

The statistical analysis was performed in IBM SPSS™ and Microsoft Excel™.

5. Conclusions

The samples tested had the abundance of *E. coli* and *K. pneumoniae*, and this seems common in several contexts. Similar results were found in Portugal, Brazil, Tanzania, India and even different subsets of the Mozambican population. Yet, there were other bacteria and they were all effectively tested for sensitivity to nitrofurantoin. According to the observations, this antibiotic should be used empirically for *M. morganii*, *E. coli* and Gram-negative bacteria. For *P. mirabillis*, *K. pneumoniae* and *C. freundii*, it is better to perform a sensitivity test before administrating the medicine. For *S. marcescens*, it is unlikely to be effective as the sole treatment.

Additional factors such as monthly and annual, subjects' sex and urine turbidity do not seem to influence significantly the sensitivity. Even the temporal variation within a cycle of five years does not seem to be significant and the medicine is still among the most effective, according to some literature. It would be useful to consider other features such as dosage and side effects.

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