Dr. Amjad Idries

Community based Monitoring of Microbiological Sensitivity test to Amoxicillin in Khartoum, Sudan 2011

Abstract:

Background: in 2009-2010 a team of experts conducted medicines quality monitoring survey. The survey showed wide range of results related to quality and effectiveness of some medicines. Among these medicines, Amoxicillin was the second generic that health professionals widely complain its effectiveness; it was the top generic that health professionals shifted their patients' treatment from it to another antibiotic. Despite this, products circulated in the market were of good quality in terms of chemical contents based on chemical analysis results. Questions were raised regarding the contribution of the wide irrational use of Amoxicillin in Sudan to these observations. This study aimed to investigate specific factors affecting the clinical outcomes of Amoxicillin in Khartoum, Sudan. **Methods**: Antimicrobial sensitivity approach was used in this study. Throat swaps were collected and tested from 102 patients used Amoxicillin to treat current illness of upper respiratory tract infections. **Results**: 64% of patients administered Amoxicillin as first treatment for their current conditions. 49% of the patients used Amoxicillin without prescription and without advice from the pharmacist. Only 23% of the patients felt a complete relief of their symptoms after 5 days of treatment. In 94 patients isolates only 51% of these isolates respond to Amoxicillin discs and the rest showed no sensitivity against Amoxicillin. **Conclusion**: Indicators form this study showed important data to inform the decision makers about the linkage between the usage of Amoxicillin with its quality of effectiveness or clinical outcomes. It indicated critical data about the microbiological response of bacteria to this drug.

Introduction:

Amoxicillin in Sudan has unique characteristics which make any study about its use and its quality is of considerable importance. These characteristics include the following: (1) currently there are 77 products registered in Sudan between different dosage forms and concentrations [1]; (2) it is the forth generic in terms of quantity (collectively exported and manufactured in the country) during the period 2006/2007 [2]; (3) it was among highly consumed products in the market, especially without prescription, which reflects the wide use of this drug in the community [2]; (4) it is a "symbol of antibiotic" in the community and it is culturally considered as one of the best accessible anti-infective drugs; (5) based on the results obtained from "unpublished" quality monitoring survey conducted in Sudan 2009-2010; Amoxicillin was the second generic that health professionals widely complain its effectiveness; and (6) based on the results from this survey; it was the top generic that health professionals shift the patient from it to other antibiotics. On the contrary for the last two observations, all assayed products in the same quality monitoring survey were found complying with standard specifications in its chemical contents (3). Based on the findings revealed from the analysis of targeted pharmaceutical products (among which "three" Amoxicillin 500 mg capsule products were tested); the results didn't respond to concerns reported about Amoxicillin products circulated in the market. Although the products have been tested were considered as of good quality, still they were clinically not effective (based on feedback of health professionals' survey). There are valid questions regarding the possibility of developing microbial resistance to this generic in Sudan or any other possible reasons. Taking into considerations the microbial resistance in such case is not an exclusive reason for therapeutic failure, as bioavailability problems and lack of appropriate use/administration may contribute also to these reported failures (4).

Looking closely to the trend of Amoxicillin use both inside and outside the health system in Sudan (prescription-based versus OTC treatment), the trend indicates the extent to which Amoxicillin is widely misused during the last decade. Studies in Europe showed clear association between higher consumption of antibiotics and the rate of microbial resistance to those antibiotics (5). This was especially noticed among the highly consumed antibiotics in the community, the same context for Amoxicillin in Sudan (6).

Basic findings about Amoxicillin from the survey:

1. The second generic that health professionals widely complain its effectiveness;
2. Top generic that health professionals shift the patient from it to another antibiotic;
3. Questions regarding possibility of developing microbial resistance to this generic in Sudan due to wide misuse;
4. No studies indicated significance instability problems;
5. Among highly consumed products in the market;
6. Expiry remaining period are satisfactory in general;
7. Significant physical appearance problems for most of the trade products

In Sudan it was generally noticed that Amoxicillin is commonly, and mainly, used for both pediatric and adult patients to treat upper and lower respiratory tract infections (U/LRTI), this usually besides other indications. Many studies confirmed the increasing prevalence of Streptococcus pneumoniae among patients with U/LRTI; beside other kinds of microorganisms (7). These facts critically raised the question about whether Amoxicillin is still an appropriate treatment for these kinds of infections. The implications of answering this question have great impact both at macro and micro levels in policy making process and at community level as well (8). Currently there is no specific study in Sudan that "linked the usage of Amoxicillin with its quality of effectiveness or clinical outcomes. Considering the large number of Amoxicillin products consumed over the country, such study will become a necessity.

World Health Assembly Resolution in 1998 urged Member States to develop measures to compact the development of microbial resistance for antimicrobials. Countries were also encouraged to develop sustainable systems to detect resistant pathogens, to monitor volumes and patterns of use of antimicrobials and the impact of control measures. For further details, World Health Assembly Resolution in 2005 urged the Member States to "ensure the development of a coherent, comprehensive and integrated national approach to implementing the strategy for containment of antimicrobial resistance" and to "monitor regularly the use of antimicrobial agents and the level of antimicrobial resistance in all relevant sectors" (6). As response to WHO recommendations about the need for monitoring process of antimicrobial resistance, this study was designed to provide necessary information to evaluate the outcomes of Amoxicillin and to inform the decision-making about the effectiveness of this drug. This was especially planned to provide evidence to inform a comprehensive policy directions to review Amoxicillin

registration, uses and monitoring in the country. The focus of this study is to investigate factors affecting the quality of clinical outcomes of Amoxicillin in Khartoum using antimicrobial sensitivity approach. This study was aimed to:

1. Investigate the incidence of microbial resistance for Amoxicillin using the microbiological assay experiment on throat clinical isolates; and
2. To compare the impact of using different Amoxicillin products on patient clinical outcomes.

Methods:

Study design

Study area: Khartoum city was divided into four geographical areas and the samples divided between these areas.

Study population: Targeted populations were selected and included based on the following criteria:

- Only patients living in Khartoum locality were considered in this study;
- Patients approaching the private pharmacies in the study area were the only targeted study population; and
- Patients complaining from symptoms of upper respiratory tract infection asking for treatment with or without a prescription.

Patients with the following criteria were excluded:

- Recent administration of sub-optimal dose of antibiotics or incomplete treatment duration within 2 weeks;
- Recent use of antimicrobial treatment during the last 3 months (especially cephalosporin derivatives);
- Under 14 years and over 60 years of age;
- Underlying diabetes;
- History of recent hospitalization

Sample size:

The purpose of sampling, in this part of the study, was to get rapid description of the current situation regarding the incidence of resistance to Amoxicillin among targeted population. In Sudan there is no drug utilization review completed recently to show any data about the quantitative use of medicines in the country (uses, quantities, sources ...ect). Accordingly, for this study the sample size was determined using a technique of total coverage of eligible patients during 10 working days. Accordingly 102 samples were collected from the study sites.

Sampling technique:

Samples were obtained from patients approaching the retail pharmacies in Khartoum city. Pharmacists working in the selected pharmacies were asked to collaborate with the study team to identify the patients receiving Amoxicillin product (based on the inclusion criteria as above). The pharmacists were trained on collecting the samples. Patients selected as part of the study received verbal information about the study and its purpose. They were informed about the reasons behind the request for biological samples from their throat using a piece of swab. Upon acquiring the verbal consent from the patients, disposable swaps were used to collect the throat sample from the patients and then inculcated into 15-20 ml of Cary-Blair transport media. The samples stored at room temperature at the collection site and then it was transported on daily basis to the Central Laboratory in Faculty of Pharmacy or Faculty Science – University of Khartoum.

Preparation of blood agar media:

The method adopted for this process was developed by Bendict L. and others with some minor modifications as needed. Blood was drawn from sheep's neck area which was shaved and swabbed with iodine (9). A venipuncture was performed in the jugular vein using a 36-inch blood collection set. The blood was collected in a Becton Dickinson Vacutainer bottle containing 1 ml of 5% SPS (Imada, India) for each 100 ml of blood to be drawn. The bottle was inverted occasionally to facilitate mixing. The blood was stored at 4 C prior to use. Sterility testing was performed by inoculating 5 ml of blood into two evacuated bottles. The bottles were then incubated at 37 C for 2 days and checked daily for visible evidence of microbial growth, prior to use. 15-ml sample of blood was used in performing a hematocrit and for sterility testing as described previously. The blood was aseptically added to the cooled agar to give a final concentration of 5%. Approximately 18 ml of blood agar was dispensed into sterile 100-mm Petri dishes and allowed to harden. All prepared plates were stored at 4 C.

Preparation of bacterial isolates:

Subcultures of collected Cary-Blair transport media was prepared by inoculating a sterile loop. Each isolate was inoculated into 2 ml of Trypticase soy broth (BBL), and the turbidity was adjusted to the barium sulphate standard used in the Kirby-Bauer method of antibiotic susceptibility testing. A loopful from this broth was streaked onto the blood agar plate for all samples. All plates were incubated at 37 C. Readings and monitoring of the growth was done in a continuous process.

Data management and analysis plan:

The data analysis process was done using mixed analysis methods according to the type of data and the tool of data collection, but in general, it was done using manual analysis and computer programs (SPSS and MS Excel).

Ethical considerations:

This study was conducted by independent professionals from the academic sector and was designed for scientific and academic purposes only. The investigators express no conflict of interest doing this research. The PI has committed prior to start the study for his responsibility to ensure the protection of the rights, safety and well-being of subjects involved in this study. This was planned to be attained, among other things, by reviewing, approving, and providing continuing review of this study protocol and the methods and material used in obtaining and documenting informed consent of the study subjects. The ethical approval has been obtained from the National Board for Ethical Review of Health Research. All study subjects after receiving the information verbally and upon agreement to taken part of the study; were asked to sign a consent form before considering him/her as study subject.

Results:

Sample characteristics:

The total samples collected for this study were 102 specimens from 100 patients with median age of 34 years (minimum of 6 years and maximum of 65 years).

% of patients		% (± SD)	Notes
% of patients using Amoxicillin as first treatment for current symptoms		64.3% (±2.6)	-
% of patients used other drug before Amoxicillin	used other beta-lactam antibiotics	44.5% (±3.2)	36 patients used other drug before the Amoxicillin
	Used other antibiotics	56.1% (±3.0)	

Of the 102 cases the following table showed the distribution of the cases according to the reason behind using the Amoxicillin:

Category of Patients	% (± SD)
Patients took Amoxicillin based on doctor's prescription	18.7 (±2.1)
Patients take Amoxicillin based on recommendation of the pharmacist	32.2 (±1.9)
Amoxicillin taken as requested by the patients themselves	49.1 (±1.8)

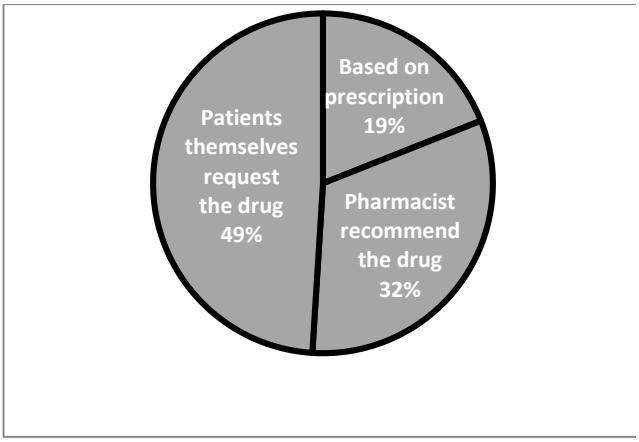

Figure 1: Classification of patients using Amoxicillin products

Follow-up of the patients:

5 days after each patient received his/her medication (appropriate Amoxicillin dosage forms including capsules or suspension with different concentrations), the study team called back all of the patients those gave their contact numbers in the pharmacies. The purpose of these phone calls was to ask the patients about the following:

- Completion of the treatment fully as prescribed;
- Feeling about the relief of the symptoms

Patients' indicator		Results
Total number of patients with contact		86 patients
No. Patients responded		79 (91.9%)
No. Patients reported completion of the treatment		58 (93.4%)
% of patients feeling about the relief of the symptoms	Complete relief	22.7% (±2.1)
	Partial relief	46.9% (±2.6)
	No relief	30.4% (±1.8)

Microbiological sensitivity:

For the purpose of this study, no typology identification was carried out for the isolates under testing. Rather the study team reported the presence or absence of microorganism growth before adding the Amoxicillin disks. In addition to that, the number of isolates with clear growth inhibition after the insertion of antibiotic disk was also reported. This data was linked to the demographic data above.

Isolates	%	Notes
% of isolates with clear growth on agar media before adding Amoxicillin disk	78.4% (±2.1)	On 22 plates no growth was detected
% of isolates with clear growth inhibition after adding the disk	48.9% (±2.1)	On 46 plates no clear growth inhibition was seen

Patients category	Total	Isolates (%)	
		Clear Growth	Growth Inhibited
Total patient isolates	94	48 (51.1%)	46 (48.9%)
Patients using Amoxicillin as first treatment	64	25 (39.1%)	39 (60.9%)
Amoxicillin taken without professional advice	49	26 (53.1%)	23 (46.9%)
Patients feel complete or partial relief	55	31 (56.4%)	24 (43.6%)
Patients feel no relief	24	2 (8.3%)	22 (91.7%)

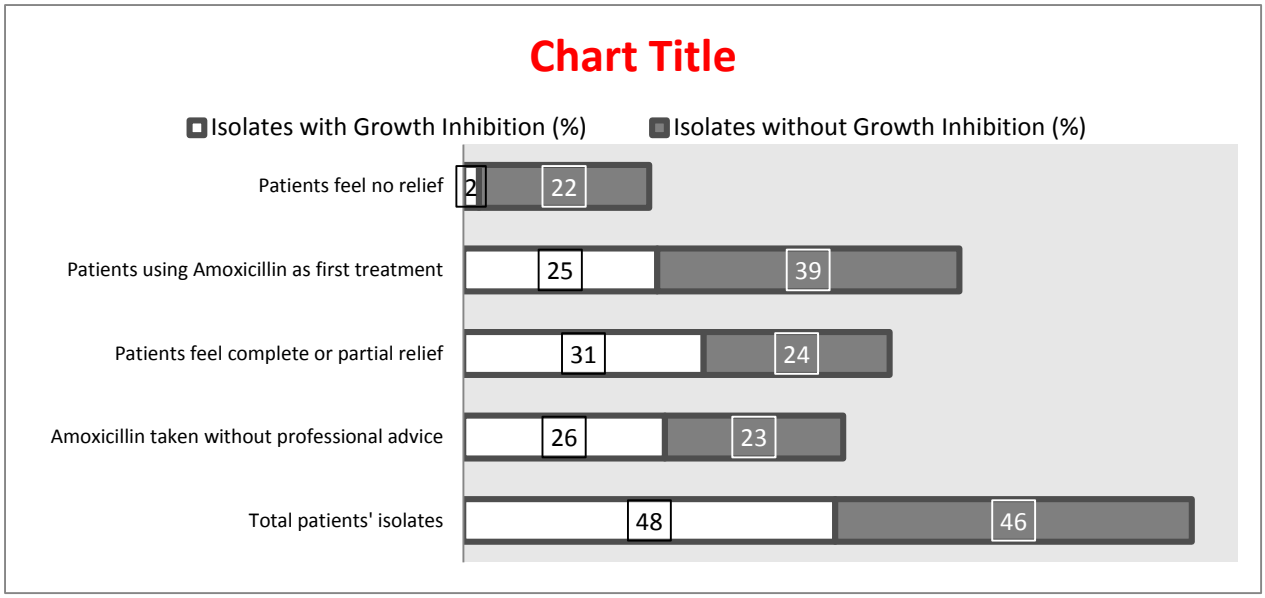

Figure 1: Summery of growth inhibition after using Amoxicillin discs

Discussion:

The above data gave clear indicators about the factors that may possibly influence the development of bacterial resistance for Amoxicillin products. That is unnecessary be linked to antibiotic use for viral respiratory infections as considered among the health professionals. In fact misdiagnosis the conditions and its symptoms lead to empirical and blind treatment of the case (in case of respiratory infections both viral and bacterial agents can cause similar clinical symptoms). Among other factors the extensive use of Amoxicillin and other similar products due to many reasons (e.g. patients' pressure on health professional to obtain rapid treatment for their disease) also played important role. This was seen clearly in the results obtained from this study.

In general, scientists agreed that the resistance of bacteria to certain antibiotic(s) is a matter of response from the bacteria towards the environmental changes due to the existence of the antibiotic "resistance as biological response". This is valid regardless whether or not the antibiotic(s) were effective against the bacteria (10).

This point supports the hypothesis about the possible contribution of microbial resistance against Amoxicillin in reported cases of ineffectiveness. Due to the wide and extensive use of Amoxicillin in the community, there is considerable possibility of emerging resistance to this drug among microorganisms that were previously sensitive to it (11). Resistance for beta lactam antibiotics (Amoxicillin is one of this

group) were traditionally being associated with the bacteria that producing beta lactamase enzyme, as this leads to deactivation of the drug by breaking the beta lactam ring in its structure "the active part of the structure" (12). Although the introduction of combination product that include Clavulonic Acid beside Amoxicillin lead to dramatic changes in overcoming this problem, still there is a wide use of Amoxicillin alone for treatment of some conditions. In comparison, it is clear that the combination mentioned before is more effective than the usage of Amoxicillin alone. However, due to some factors including the economic aspects and the shape of the market in Sudan still we can observe the wide use of Amoxicillin alone for different kinds of infections (especially in U/LRTI).

Based on the results generated from this study it becomes very obvious the needs to develop structured protocol to assess the quality of Amoxicillin and other similar products using microbiological assay approach. The step of this assay should be the starting point for in-depth analysis that aims to identify the possible causes for the treatment failure in patients using Amoxicillin and other similar products.

Conclusion:

This research is small scale project and due to that still there are some limitations identified in its development. Still the findings showed statistically significant outcomes for measures to degree of the problem but further research and in-depth studies were needed in this area to be done in the future. The responsible authorities should develop practical mechanism to monitor the use of antimicrobial agents and the level of antimicrobial resistance in all relevant sectors.

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