AN EVALUATION OF QUALITY AND BRANDS OF AMOXICILLIN FORMULATIONS MARKETED IN NAIROBI CITY COUNTY, KENYA

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NOVEMBER, 2019
DECLARATION

I hereby declare that this is my original work and has not been presented for the award of a degree or examination at any other institution.

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DEDICATION

This thesis is dedicated to my loving Family.
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ABBREVIATIONS AND ACRONYMS

AAOS: American Academy of Orthopedic Surgeons
ADA: American Dentistry Association
AHA: American Heart Association
API: Active Pharmaceutical Ingredient
BP: British Pharmacopoeia
CE: Capillary Electrophoresis
GMP: Good Manufacturing Practice
HPG: $p$- hydroxyl-phenylglycine
HPGM: hydroxyl-phenylglycine methyl ester
IP: International Pharmacopoeia
IR: Infrared Spectroscopy
KAPI: Kenyan Association of Pharmaceutical Industry
LC MS/MS Liquid Chromatography Tandem Mass Spectroscopy
NAMA: N-acetyl muramic acid
NAG: N-acetyl glucuronic
NIR: Near Infrared
NQCL: National Quality Control Laboratories
ODS: Octadecylsilyl
PBPs: Penicillin Binding Proteins
PGA: penicillin G acylase
PTFE: polytetrafluoroethylene
LC: Liquid Chromatography
TLC: Thin Layer Chromatography
USP: United States Pharmacopoeia
UV/VIS: Ultra Violet/Visible
ABSTRACT

Antibiotics are among the most counterfeited anti-infectious medicines in developing countries due to high demand. Amoxicillin is among the most prescribed, affordable and easily accessible antibiotic in Kenya. It is a broad-spectrum antibiotic hence commonly used for chemotherapy. Administration of poor-quality medicine possess the potential risks of treatment failure, emergence of resistance, side effects, and/or death to patients. This study sought to determine the quality and identify the various brands of amoxicillin and its combination amoxicillin/clavulanic acid marketed in Nairobi County. Nairobi is the capital city of Kenya, gateway for imports and exports and the headquarters to most of the pharmaceutical distributors and wholesalers. Ten wards in Nairobi County representing different socio-economic settings were purposively sampled for the study. A detailed questionnaire was used to collect background data on brands of amoxicillin and amoxicillin/clavulanic acid in the market. A total of 106 different brands of amoxicillin and amoxicillin/clavulanic acid formulations were found in the market, 85 were imports while 21 were locally manufactured. Fifty-three samples were analyzed with reference to the United States Pharmacopoeia monograph. Amoxicillin and clavulanic acid content for oral suspensions were determined immediately after reconstitution and 7 days thereafter to determine their stability during prescription period. On day seven 23.1% (3 out of 13) of amoxicillin and 66.7% (8 out of 12) amoxicillin/clavulanic acid oral suspensions presented levels below recommended limits. Potency determination for amoxicillin capsules and amoxicillin/clavulanic acid tablets showed 33.3% (2 out of 6) and 13.6% (3 out of 22) failure rate. For capsule and tablets dissolution tests, there was 17.9% (5 out of 28) failure rate. Overall, 30.2% of the drugs analyzed failed to comply with pharmacopoeia limits. It is therefore worth noting that quality of some amoxicillin formulations in Nairobi County is not to standard. This affirms the need for regular post market surveillance to inform on the situation of antibiotics quality in the Kenyan market.
CHAPTER ONE

INTRODUCTION

1.1 Background Information

Over the past decade, increased public awareness on drug quality has been assessed in terms of counterfeit and or substandard (HealthTech Report, 2014). The WHO estimates that up to 10.5% of the world’s pharmaceutical trade consists of counterfeits with 25.0% of the drugs being consumed in most developing countries (WHO, 2010). Substandard and counterfeit medicines problem is complex but an important and critical global health concern (WHO, 2017). Counterfeit drugs are products deliberately and fraudulently produced and /or mislabeled with respect to identity and/or source to make it appear to be a genuine product (WHO, 2003). Substandard drugs are genuine drug products that upon laboratory testing do not meet set quality specifications and/or it claims to comply with (WHO, 2003).

All pharmaceutical products are predisposed to compromised quality; both innovator and generic medicines ranging from expensive products (cancer medicines) to very inexpensive products (painkillers) can be falsified (WHO, 2018a). Anti-infectious agents particularly antibiotics and anti-parasitic agents being the most counterfeited products in developing countries (Johnston and Holt, 2014). Antibiotics, account for 28.0% of global counterfeits with the most commonly counterfeit antibiotic formulations being oral administrative (77.0%), and injected formulations (17.0%) (Delepierre et al., 2012). Approximately 1 in 10 medicines in low and middle income countries is counterfeit or substandard (WHO, 2018a). The scale keeps broadening with use of internet to sell medicines, as about 50.0% online purchases from sites that conceal their physical address being counterfeit (WHO, 2017). Majority of developing countries struggle with
enforcing regulations on medicine distribution and sustaining drug quality of therapeutic drugs majorly because of the disease burden, weak technical capacity and supply chain management (WHO, 2017).

1.2 The quality of antibiotics in Kenya

In Kenya, there has been deliberate effort to carry out studies on quality of drugs in the market since the early 1980’s with reports of presence of compromised quality (Kibwage et al., 1992; Kamau et al., 2003; Kibwage et al., 2008; Nguyo et al., 2008; Abuga K, et al., 2013; Wafula et al., 2017) A random survey by the National Quality Control Laboratories (NQCL) and the Pharmacy and Poisons Board (PPB) found that almost 30.0% of drugs sold in the country are counterfeit (WHO, 2006). The Kenyan Association of Pharmaceutical Industry (KAPI) reported that counterfeit and substandard pharmaceutical products account for approximately Kenya shillings 9 billion ($100 million) annually in sales. This figure corresponds to 20-25% of the total legit pharmaceutical market (Elaine, 2011).

According to the National Quality Control Laboratory (NQCL) reports, the failure rate for antibiotics was reported at 24.3% in the year 1996 - 2001, 9.4% in 2004-2005 and 17.3 % in 2006-2007 (Kibwage, 2008). Kamau et al., (2003) in a study on 57 amoxicillin formulations in the Kenyan market recorded 11.0% failure rate in the products. In yet another study that analyzed 43 ampicillin products, 21.0% had lower API than the label claims (Thoithi et al., 2001). Another research evaluated metronidazole tablets by different manufacturers in the Kenyan market. Two products failed the dissolution test releasing only 46.8% and 45.8% of
drugs in 40 min. Drug release from the tablets was found to vary between batches (Kibwage et al., 1991).

It is evident from the quality studies conducted over the last three decades on various antibiotic drugs in Kenya that drug failure rates still remain unacceptably high. In Kenya drug imports are customs and duty free and importation is open to anybody who is legally allowed to handle medicines (Act of Parliament Cap 472, 2010). This gap can be easily exploited by unscrupulous persons who deal in counterfeit and substandard drugs.

1.3 Statement of the problem

The quality of drugs is presently receiving massive renewed international attention. According to the WHO, 50.0% of medicines being sold in Africa are thought to be counterfeit or substandard (WHO, 2017). This issue has significant health and economic consequences, with reports of deaths in Sub-Saharan Africa of approximately 140,000 children annually from pneumonia and malaria as a result of counterfeit and substandard treatments (WHO, 2018a). This threatens global health security and universal health coverage under Sustainable Development Goals 3.8, to achieve universal access to safe and effective essential medicines (WHO, 2018a). In Kenya the magnitude of substandard / or counterfeit antibiotics is unknown, yet the issue of poor quality drugs is extensively discussed in the media and respective ministries (Sambira, 2013; Philippa, 2015; Lukulay, 2016; Nation Team, 2016; Mareb, 2017; Gatonye, 2018; Kajilwa, 2018;). This indicates the need for information on quality of such commonly prescribed antibiotics in this case amoxicillin to inform the public and policy.
1.4 Justification of the study

In Kenya antibiotics is one of the most important but commonly overprescribed form of medicine therapy (GARP, 2011). Prevalence of diseases that depend on antibiotic therapy treatment such as pneumonia, tuberculosis and diarrheal infections is also affected by use of low quality antibiotics (HealthTech Report, 2014). Generic products are much cheaper than their innovator versions as generic manufacturers do not incur the initial investment costs for new drug development (Kelesidis et al., 2007). This has resulted to the number of multinational pharmaceutical companies actively involved in antibiotic drug discovery research reducing from 18 in 1990 to only 4 (AstraZeneca, Novartis, GlaxoSmithKline (GSK), and Sanofi-Aventis) in 2011 (Butler et al., 2013).

Most public health literature in Kenya gives a great deal of attention to the misuse of antibiotics but comparatively little work, however discusses the role of drug quality in encouraging bacterial resistance (GARP, 2011). Consequently, preserving antibiotics is imperative and depends on maintaining drug quality as much as on encouraging rational use. This study therefore will provide information on the various brands and determine the quality of amoxicillin formulations marketed to the public within Nairobi County.
1.5 Hypothesis

The quality of amoxicillin in Nairobi retail and private hospital pharmacies is not acceptable as per monographs outlined in the United States pharmacopeia (USP).

1.6 Objectives

1.6.1 General Objective

To investigate the quality of selected brands of amoxicillin in Nairobi’s retail and private hospital pharmacies.

1.6.2 Specific Objectives

i. To establish the available brands of amoxicillin formulations in retail and private hospital pharmacies within Nairobi County.

ii. To determine the amounts of active pharmaceutical ingredient (API) of solid dosage forms and dry oral suspensions of amoxicillin formulations within Nairobi’s pharmacies.

iii. To determine dissolution profile of selected solid dose forms of amoxicillin available in Nairobi’s pharmacies.

1.7 Significance of the study

The data obtained will inform government and policy makers on the need to enhance anti-counterfeit, medicine quality control efforts and improve on reporting channels. In addition, there is need to invest in conducting public information campaigns and routine surveys of medicine quality in the Kenyan market. Majorly because, countries with weak regulatory
oversight and law enforcement attract illegitimate manufacturers, while countries with strict law enforcement deter them (WHO, 2018a).

1.8 Scope and limitations of the Study
This study focused only on amoxicillin and amoxicillin/clavulanic acid combinations in selected pharmacies within Nairobi County, Kenya. Only content determination, uniformity and dissolution tests were conducted. Therefore, “quality” in this study refers only to the acceptable amount and uniformity of the active ingredient in terms of the ranges specified by the USP.
CHAPTER TWO

LITERATURE REVIEW

2.1 Antibiotics

Antibiotics can broadly be defined as chemical that selectively inhibits an infectious biological agent but causes minimum damage to the host; thus this includes antibacterial, anticancer, antifungal, antimalarial and antiviral antibiotics (Butler et al., 2013). Antibiotic that kill bacteria are termed as bactericidal and those that inhibit bacterial growth are termed as bacteriostatic.

The most common classification scheme of antibiotics are based on their molecular structures, mode of action and spectrum of activity (Etebu and Arikekpar, 2016). Classification as shown in Table 2.1 based on chemical or molecular structures include Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones (Etebu and Arikekpar, 2016).

2.2 Advantages of antibiotics

The reduction of disease burden globally from acute bacterial infections that caused high mortality before their introduction cannot be disputed. Mortalities from infections such as bacterial meningitis, endocarditis, and acute osteomyelitis has been reduced to 8-20%, 20% and less than 1% currently (Lozano et al., 2012; Bartlett, 2014). Up to 80% of new cases of Pseudomonas aeruginosa infections has been eradicated using inhalants and oral antibiotics, with reduced need for additional intravenous antibiotics (Langton and Smyth, 2014).
Table 2.1: Classification of antibiotics based on their molecular structures (Etebu and Ariekkar, 2016).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Some Examples</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-Lactams</td>
<td>(i) Penicillin derivatives (penams such as amoxicillin, Flucloxacillin); (ii) Cephalosporins (cefaolin, cephalexin); (iii) Mono-bactams (Aztreonam, Nocardicin); (iv) Carbapenems (meropenem, Doribax)</td>
<td>Inhibit cell wall biosynthesis</td>
</tr>
<tr>
<td>Macrolides</td>
<td>(i) Azithromycin (ii) Erythromycin (iii) Clarithromycin (iv) Spiramycin</td>
<td>Inhibit bacteria protein synthesis</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>(i) Doxycycline (ii) Minocycline (iii) Oxytetracycline (iv) Tigecycline</td>
<td>Inhibit synthesis of protein by bacteria, preventing growth</td>
</tr>
<tr>
<td>Quinolones</td>
<td>(i) Ciprofloxacin (ii) Levofloxacin (iii) Trovafloxacin</td>
<td>Interfere with bacteria DNA replication</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>(i) Streptomycin (ii) Neomycin (iii) Kanamycin (iv) Paromomycin</td>
<td>Inhibit synthesis of protein by bacteria, leading to cell death</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>(i) Prontosil (ii) Sulfanilamide (iii) Sulfadiazine (iv) Sulfisoxazole</td>
<td>Prevent growth and multiplication of bacteria</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>(i) Vancomycin (ii) Teicoplanin</td>
<td>Inhibit cell wall biosynthesis</td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td>(i) Linezolid (ii) Tedizolid (iii) Posizolid (iv) Cycloserine</td>
<td>Inhibit synthesis of protein by bacteria, preventing growth</td>
</tr>
</tbody>
</table>

Antibiotic therapy in exacerbations of chronic pulmonary disease has been successful and decreased respiratory morbidity has been documented from use of effective antibiotics (Langton and Smyth, 2014). General physicians have been able to successfully treat urinary tract
infections caused by uropathogens; *Escherichia coli, Staphylococcus saprophyticus, Klebsiella pneumonia and Proteus mirabilis* (Baroud *et al.*, 2013). Early prescription of antibiotics have significantly lowered surgical sepsis (Bartlett, 2014).

Antibiotics as a prophylaxis are also been successful for bacterial meningitis in high risk patients (Hoffman and Weber, 2009). In cases of viral induced respiratory tract damage, antibiotics are prescribed to prevent secondary bacterial infections (Hoffman and Weber, 2009). Such as in prevalence of *Staphylococcus aureus* infection and other secondary bacterial in cystic fibroids patients with presumed acute viral respiratory infections oral flucloxacillin is used (Langton and Smyth, 2014). Ciprofloxacin is used in prevention of *Pseudomonas*-associated deterioration in patients with chronic *Pseudomonas aeruginosa* infections (Langton and Smyth, 2014). Prevention of urinary tract infections has been effective with continuous low dose antibiotic prophylaxis (Baroud *et al.*, 2013).

Surgical site infection has been reduced substantially by use of antibiotic prophylaxis in high risk patients (Cohen *et al.*, 2017). In general and orthopedic surgery, especially when a prosthetic is being introduced, broad spectrum Cephalosporins are widely used to counter anticipated organisms (Sollecito *et al.*, 2015). In transplant recipients especially for cardiac and pulmonary; prophylactic peri-operative antibiotic minimizes the incidences of infections (Cohen *et al.*, 2017) and such patients with a history of tuberculosis mostly receive prophylactic isoniazid (HealthTech Report, 2014). Patients on immunosuppressive therapy are put on long-term prophylaxis of antibiotics (Walker *et al.*, 2010).
2.3 Disadvantages of antibiotics

Despite the significance achieved from antibiotics the disadvantages have also been noted. Side effects such as hypersensitivity; high doses of penicillin may cause haemolytic anaemia due to cytotoxic antibodies (Eppes and Childs, 2002). Drug interactions and toxicity has been associated with combination of aminoglycoside with certain Cephalosporins (Vlassoff, 2007). Drugs that induce liver enzymes such as rifamycin causes low oestrogen pill to lose its contraceptive effect (Simmons et al., 2018). Alteration of normal flora of the body hence allowing for colonization by the multiplication of resistant and opportunistic pathogens; especially for broad spectrum antibiotics (Salvo et al., 2007). These may cause fungal and systematic infection in high susceptible patients such as transplant patient on immunosuppressive therapy or secondary infection like candida vaginitis in healthy patients (Walker et al., 2010).

Antibacterial resistance is similarly a major shortcoming and is due to bacterial genetic alterations thus metabolizing the antibiotic such as alterations in cell wall permeability, receptor site and influence of environmental factors at infection sites (Baroud et al., 2013). Methicillin resistant \textit{Staphylococcus aureus} (MRSA) have a thick cell wall that impairs diffusion of antibiotics to site of action. A multifactorial range of independent risk factors for MRSA have been documented such as hemodialysis, immunosuppression, extended in-hospital stays and long term care facilities, advanced age, inadequacy of antimicrobial therapy, indwelling devices, insulin-requiring diabetes, and decubitus ulcers, among others (Contreras-Martel \textit{et al.}, 2011). MRSA treatment using Vancomycin has been widely used but has resulted in adverse effects
over the years (Thakuria and Lahon, 2013). Glycopeptide antibiotic, Teicoplanin has shown better activity and longer half-life with lesser side effects in treatment of MRSA (Tian et al., 2011a). Synthetic oxazolidinones; Linezolid and tedizolid are also effective in MRSA treatment but restricted due to adverse effects in bone marrow density and mitochondrial toxicity (Herrmann et al., 2008).

Multiple factors contribute to the increase in the prevalence of antibiotic resistance globally. In developing countries, resistance has been faulted on misuse, poor monitoring of antibiotic use, lack of surveillance of resistance, ease of availability and most recently poor quality of available antibiotic being factored (WHO, 2018a). A major concern being that many developing countries do not have the necessary quality assurance mechanisms to monitor standards of manufacturing and importation, transportation and storage, counterfeits and relabeling of expired products (GARP, 2011).

2.4 Beta–Lactams antibiotics
Beta-lactams ($\beta$-lactams) are a family of antibiotics with a four membered cyclic amine (lactam) and nitrogen atom attached to the $\beta$-carbon atom relative to the carbonyl (1) (Hubscherlen, 2007). They are the most widely produced and used antibacterial drugs globally since their first clinical trials in 1941. $\beta$-lactams consumption accounts for 65.0% of the total global antibiotic market (Thakuria and Lahon, 2013), majorly because they work extremely well against a wide variety of bacteria while exerting little toxicity towards mammalian cells. $\beta$-lactams are broadly divided into several classes based on their structure and function; the principal ones being
penicillin’s (2), cephalosporins (3), carbapenems (4), and monobactams (5) (Hubschwerlen, 2007).

2.5 Penicillin

Penicillin’s consist of a bicyclic ring, where the β-lactam ring is fused to a five membered ring with a thioether group and an amine group in the 1\textsuperscript{st} and 4\textsuperscript{th} positions (6). The penicillin nucleus consists of 6-aminopenicillanic acid (6-APA), (6) which can be modified at the R\textsubscript{1} position to introduce a range of different side chains of varied chemical properties such as in amoxicillin and ampicillin. Amoxicillin is classified as broad-spectrum amino-penicillin (Heesemann, 1993).
2.6 Broad spectrum beta-lactams

Originally \( \beta \)-lactams were solely active against gram-positive bacteria, since its cell wall outer surface is composed of only peptidoglycans layers. Hence action of the penicillin binding proteins (PBPs) by the \( \beta \)-lactams is easier as shown in Figure 2.1 (Silhavy et al., 2010). The cell wall of gram-negative organisms in addition to peptidoglycans layers has a bilipid layer membrane that has porin channels on the cell wall. This bilipid layer prevents passage of antibiotics except for broad / extended spectrum since they can permeate through porin channels. Hence extended spectrum \( \beta \)-lactams have been modified to make use of import porins in the cell membrane of gram-negative organisms (Heesemann, 1993; Hubscherlen, 2007).
Figure 2.1: Structural difference of Gram Negative and Positive bacteria cell walls (Silhavy et al., 2010).

2.7 Mechanism of action of Beta Lactams

Bacteria have a cytoplasmic membrane surrounded by a periplasmic space that is enclosed by a peptidoglycan layer and finally the outer membrane. The peptidoglycan layer is a cross linked polymer providing structural rigidity to the organism allowing it to survive in hypertonic media. It is composed of repeating units of N-acetyl glucuronic (NAG) and N-acetyl muramic acid (NAMA). Cross links between the chains are provided by peptides, for example glycine pentapeptide in staphylococci which bridges the gap between D-alanyl-D-alanine peptides attached to NAMA moieties in the respective chains (Baldwin and Schofield, 1992).

The cross-linking reaction is catalyzed by a peptidoglycan trans-peptidase located in the cell membrane. This class of trans-peptidase is called penicillin binding proteins (PBPs). A critical part of the process is the recognition of the D-alanyl-D-alanine sequence of the NAMA peptide side chain by the PBPs. The β-lactam ring in penicillin (2) closely resembles the configuration of
D-alanyl-D-alanine (7), thus intact β-lactam antibiotics can serve as a substrate for the transpeptidase (Baldwin and Schofield, 1992). Once they have combined with the trans-peptidase enzymes; they remain bound regardless of the drug concentration on the medium. This disrupts the cross linking process, thus inhibiting cell wall formation (Thakuria and Lahon, 2013).

2.8 Bacterial resistance to beta lactams

Bacteria have many methods with which to combat the effects of β-lactam type drugs. Resistance to β-lactams may occur in four various ways. First, access to PBPs in gram-negative bacteria might be reduced or eliminated by mutations in porin channels. This phenomenon predominantly affects highly water-soluble β-lactams. For example, in Enterobacteriaceae; *Pseudomonas spp.* and *Acinetobacter spp.* reduction in porin expression significantly contribute to resistance to newer β-lactams such as carbapenems and cephalosporins, to which resistance is usually facilitated/mediated by enzymatic degradation (Baroud *et al.*, 2013). The reduced expression or absence of the Opr D porin of *Pseudomonas aeruginosa* reduces the permeability of the cell wall to carbapenems (Langton and Smyth, 2014).

The second method of development of resistance is modification of PBPs, where resistant strains produce PBPs with extremely low affinity for β-lactams which catalyze trans-peptidation
reaction even in presence of high concentrations of β-lactams. This has been observed in some gram-negative bacteria such as methicillin resistance *Staphylococcus aureus* (MRSA) and some gram-positive bacteria such as *Streptococcus pneumoniae* (Contreras-Martel *et al.*, 2011). This mechanism generally affects the class-B PBPs involved in cell division, which is one of the main targets of β-lactams in bacteria organisms. For example modifications are encountered in *Streptococcus pneumoniae* PBP2x (Contreras-Martel *et al.*, 2011), *Neisseria gonorrhoeae* PBP2 (Brannigan *et al.*, 1990) and *Haemophilus influenza* PBP3 (Dabernat *et al.*, 2002). In addition, horizontal gene transfer also contributes to dissemination of resistance that occurs either through conjugation, transformation or transduction. For example, in *Staphylococcus aureus*, resistance is disseminated via natural transformation (Hakenbeck, 1999) and resistance in MRSA originates from transduction of the *mec A* gene, coding for a methicillin resistant PBP2a protein, into the chromosome of *Staphylococcus aureus* (Barlow, 2009).

The third and most abundant mechanism is the production of hydrolyzing enzymes called β-lactamases. These enzymes are serine protease enzymes that cleave the β-lactam ring by opening the amide bond. This system of resistance is very efficient as these enzymes are secreted out of the cell wall in gram-positive bacteria and in the periplasmic space in gram-negative bacteria. The enzymes’ affinity for β-lactams is greater than that for PBPs (Contreras-Martel *et al.*, 2011). There are 927 β-lactamases from 24 different β-lactamases classes listed on the authoritative website managed by George Jacoby at Lahey Clinic (http://www.lahey.org/studies/). Most β-lactamases open the β-lactam ring similar to PBPs but hydrolysis rate by β-lactamases is quicker than that of PBPs. This explains the high efficacy of β-lactamases (Van Bambeke *et al.*, 2003). For example, prolonged administration of β-lactam antibiotics could lead to emergence of
*Pseudomonas aeruginosa* β-lactamases resistant to multiple β-lactams, eventually leading to treatment failure and patient death (Baroud *et al.*, 2013).

The fourth mechanism for drug resistance is the differential expression of efflux mechanisms whereby, energy dependent efflux pumps due to their poly-substrate specificity, on recognizing an antibacterial compound as a potential substrate, force it out. Genes encoding this class of efflux proteins are located either on chromosomes or plasmids (Masuda *et al.*, 1999). Efflux pumps also cause acquisition of additional resistance mechanisms by lowering intracellular antibiotic concentration and promoting mutation accumulation (Masuda *et al.*, 2000). For example efflux system AcrAB-ToIC in *Escherichia coli* actively export β-lactams among other antibiotics (Touzé *et al.*, 2004), this has also been reported in *Pseudomonas aeruginosa* efflux systems MexAB-OprM and MexXY-OprM (Masuda *et al.*, 2000).

### 2.9 Development and Synthesis of Amoxicillin

The original fermentation derived Penicillin (8) faced failure majorly because the β-lactam ring could react in some neutral or basic solutions and was acted on by β-lactamase, an enzyme produced by some bacteria that renders it inactive. Thus, there was need to improve the chemical structure of penicillin to increase its acid stability, prevent β-lactamase resistance and produce broad spectrum drugs (Diender *et al.*, 1998). This was achieved in 1961 with the synthesis of ampicillin (9) and, later in 1970 with amoxicillin; which is a congener of ampicillin, hydroxylation of the phenyl side chain in amoxicillin being the difference in their structures (Gonçalves *et al.*, 2003).
2.9.1 Synthesis of amoxicillin

Bio-catalytic/enzymatic synthesis of β-lactam antibiotics is favorable for mass production as it is economically and ecologically friendly technology. This synthesis occurs in lower energy demands which are aqueous environments, at neutral pH and around room temperatures. In addition, bio catalysis provides high stereo selectivity and minimal need for extensive protection of functional groups of reagents and their activation (Gonçalves et al., 2003).

Bio catalytic synthesis of β-lactams (in this case amoxicillin) can be accomplished either via thermodynamically or kinetically controlled reactions (Diender et al., 1998). The most frequently applied strategy for enzymatic synthesis is a kinetically controlled reaction. This is achieved from hydroxyl-phenylglycine methyl ester (HPGM) and 6-amino penicillanic acid (6-APA) catalyzed by penicillin G acylase. The Kinetically controlled enzymatic synthesis of amoxicillin is shown in schematic diagram as shown in Figure 2.2 (Gonçalves et al., 2003).
Penicillin G acylase was first introduced in 1960 and is industrially produced from recombinant \textit{Escherichia coli} systems. Penicillin G acylase from \textit{Escherichia coli} is active at pH range 6-8 and requires that the phenylglycine carboxyl group be protonated while, at the same time, the amino group of the $\beta$-lactam nucleus be neutral, available for nucleophilic interactions (Gonçalves \textit{et al.}, 2003). Penicillin G acylase catalyzes the synthesis of HPGM and APA, this reaction is irreversible, but amount of product could reduce if amoxicillin hydrolysis increases. Undesired reactions that compete with product formation are: substrate hydrolysis, (Hydrolysis 1) and product hydrolysis (Hydrolysis 2) that result in $p$-hydroxyphenyl glycine (HPG) production (Alemzadeh \textit{et al.}, 2010).

![Chemical diagram](image)

Figure 2.2: Kinetically controlled enzymatic synthesis of amoxicillin (Gonçalves \textit{et al.}, 2003).
2.9.2 Physical and chemical characteristics of Amoxicillin

Amoxicillin (10) was first introduced in the early 1970’s and has found regular use as a broad-spectrum antibiotic. It is an acid stable, semi synthetic drug belonging to \(\beta\)-lactams antibiotics amino-penicillin class. It is mostly presented as amoxicillin trihydrate (off white) and amoxicillin sodium (white or slightly pink amorphous and very hygroscopic with slight sulphurous odor) salt (USP40-NF35, 2017b). Amoxicillin degrades with increase in temperatures both in sealed and open containers. Under controlled humidity amoxicillin undergoes first order degradation (Deshpande et al., 2004). Solubility of amoxicillin increases with increase in pH and reported pKa values of amoxicillin are 2.67, 7.11 and 9.55 at 37°C with lowest solubility at pH range of 4-6 (Rolinson et al., 2007).

![Chemical structure of Amoxicillin](image)

2.9.3 Amoxicillin /Clavulanic acid drug combination

Amoxicillin is predisposed to degradation by \(\beta\)-lactamase producing bacteria, thus it’s combined with a \(\beta\)-lactamase inhibitor such as clavulanic acid/clavulanate. Clavulanic acid, \(Z\)-(3R, 5R)-2-((3-hydroxyethylidene) clavam-3-carboxylate, is a \(\beta\)-lactam compound isolated from *Streptomymyces clavuligerus* fermentation; a species of a gram-positive bacterium (Saudagar et al., 2008). It is a potent inhibitor of plasmid mediated \(\beta\)-lactamase, including those produced by *Haemophilus influenza*, *Staphylococcus aureus*, *Neisseria gonorrhea*, and *Bacteroides fragilis*.
that are frequently involved in the resistance to β-lactams such as penicillin’s and cephalosporin’s (Finlay et al., 2003). Clavulanic acid is a β-lactam structurally related to penicillins and has very weak antimicrobial activity (Saudagar et al., 2008).

It acts as a pseudo-substrate, inhabiting the active site of the β-lactamase for adequately long time to inhibit degradation of co-administered β-lactam antibiotics. Clavulanic acid when available at very high concentrations, binds irreversibly to the β- lactamase through a complex mechanism forming a stable acyl-enzyme complex (Finlay et al., 2003). Clavulanic acid hence enhances the activity of β-lactam compounds, thus amoxicillin/clavulanic combination is clinically used and bacteriologically works better than amoxicillin alone (Finlay et al., 2003).

2.9.4 Pharmacokinetics of Amoxicillin

Amoxicillin has broad antibacterial spectrum and high oral bioavailability (70-90%) with highest plasma levels occurring within 1-2 hours of intake. It is dose dependent and its bioavailability is up to three times greater than that of ampicillin after comparable oral doses (Badraddin et al., 2011). It therefore has extensive clinical use. Amoxicillin is well absorbed from the gastrointestinal tract, it’s distributed to many tissues including liver and lungs (Badraddin et al., 2011), muscles, bile (Kiss et al., 1981), ascetic, pleural and synovial fluids, and ocular fluids (Carlqvist and Westerlund, 1979).

It also accumulates in the amniotic fluid and crosses the placenta, but penetrates poorly into the central nervous system unless there is inflammation, quantity approximately between 10-60% of the amount found in serum (Nahum et al., 2006). Extremely minimal amounts of the drug may be found in the aqueous humor and tears in eyes, sweat and saliva (Badraddin et al., 2011).
Approximately 17-20% is bound to human plasma proteins, predominantly albumin (Eppes and Childs, 2002).

Apparent volume of distribution of amoxicillin ranges from 0.27-0.32 l/kg in adults with normal renal function. Excretion of amoxicillin is primarily renal, and greater than 80.0% of which 50-70% of the administered doses are recoverable in urine unchanged, and is also secreted in milk (Chulavatnatol and Charles, 1994). Amoxicillin terminal half-life of elimination is 1-1.5 hours in adults and approximately 19-33% of the drug is metabolized into penicilloic acid-a biologically inactive metabolite (Eppes and Childs, 2002).

2.9.5 Pharmacology of Amoxicillin

Amoxicillin is effective against a wide range of infections caused by a variety of gram positive and gram-negative bacteria in both humans and animals. Over the past decade, amoxicillin has been reported useful for the treatments of infections of the middle ear (otitis media) (Calhoun and Hokanson, 1993), tonsils (tonsillitis pharyngitis), throat and larynx (laryngitis), pharynx (pharyngitis) and bronchi (bronchitis) (Imoisili, 2008), lungs (pneumonia) (Curtin-Wirt et al., 2003), urinary tract infections (Fernandez-Torres et al., 2010), skin infections and gonorrhea (Eppes and Childs, 2002).

Published reports further suggest amoxicillin as a potential drug for treatment of Chlamydia trachomatis (Kacmar et al., 2001), typhoid fever (Imoisili, 2008), early lime disease (Jyothi et al., 2010), erythema migraines and erythema migraines borreliosis (Hoffman and Weber, 2009), mucopurulent cervicitis (Marrazzo and Martin, 2007), acute maxillary sinusitis (Sande and
Gwaltney, 2004), gastritis and peptic ulcers (Goodwin et al., 1986) and meningitis conditions (Hoffman and Weber, 2009).

American Heart Association (AHA), American Dental Association (ADA) and new recommendation by American Academy of Orthopedic Surgeons (AAOS) suggested that it can be used as prophylaxis against bacterial endocarditis in patients with prosthetic joint replacements and in dentistry (Kotzé, 2009).

2.10 Adverse effects of Amoxicillin and Amoxicillin/Clavulanic Acid combination

β-lactams are normally safe drugs and serious adverse effects are rare and allergies are over diagnosed. A study to create a database documenting side effects of drugs conducted within the WHO programme for International Drug Monitoring from January 1988 to June 2005, identified 37,906 reports. Reports related to amoxicillin alone were 1,095 and 1,088 to amoxicillin combination (amoxicillin/clavulanic acid). The percentage of skin reactions were high for both amoxicillin alone and amoxicillin/clavulanic acid combination; 82.0% and 76.0% respectively, conversely the percentage of gastrointestinal, hepatic and hematological reactions was higher for amoxicillin/clavulanic acid combination (13.0%, 4.0% and 2.0% respectively) than for amoxicillin alone (7.0%, 1.0% and 1.0% respectively) (Salvo et al., 2007).

Amoxicillin/clavulanic acid combination is associated with a higher risk of Stevens - Johnson syndrome (Limauro et al., 1999), purpura; inflammation and bleeding of small blood vessels (Berg and Hahn, 2001) and hepatitis (Zaidi et al., 2003), with eventual systematic dysfunction than amoxicillin alone.
2.11 Quality control tests for Amoxicillin and Clavulanic Acid

Various methods of analysis of amoxicillin trihydrate and its combination amoxicillin/ clavulanic acid have been described in the official pharmacopoeia and in published scientific journals. The pharmacopoeia is a public book describing drugs chemicals and medicine and the recommended procedures of analysis and specifications for the determination of pharmaceutical substances, excipients and dosage forms. It consists of a general part (tests, methods and general requirements) and a specific part in the form of monographs for pharmaceutical substances. Some international used pharmacopoeia includes; United states pharmacopoeia (USP), British Pharmacopoeia (BP), International Pharmacopoeia (Ph. Int.), European Pharmacopoeia (Ph. Eur.), Indian pharmacopoeia (IP), Chinese pharmacopoeia (CP) and the Japanese pharmacopoeia (JP). The USP and BP are the most commonly used in Kenya (Kenya PPB, 2009).

2.11.1 Identification test for active pharmaceutical ingredient

Identification test is necessary to ensure that the sample has the relevant active ingredient. The BP 2017 describes the use of infrared absorption spectroscopy where 0.1 g/ml of amoxicillin sample in water are prepared and agitated for 5 minutes, filtered and residue washed with absolute ethanol and then with Ether and dried in pressure not exceeding 0.7 kPa for an hour. The infrared spectrum of sample should be in concordance with the amoxicillin trihydrate reference standard spectrum. It also describes TLC method that uses silica gel salinized plate (Merck salinized silica gel 60 F254s) RP-18; mixture acetone and ammonium acetate solution 15.4% w/v solution adjusted to pH 5.0 using glacial acetic acid (10:90) and sample prepared to 0.25% w/v of amoxicillin in sodium hydrogen carbonate solution (BP, 2017).
Similarly, the USP (2017) describes a TLC method that uses plates coated with 0.25 mm layer of silica gel; mixture, methanol, chloroform, water and pyridine (90:80:30:10) as the solvent system. The sample and USP amoxicillin reference standard prepared by weighing each 4 mg per ml in 0.1N hydrochloric acid; Plate developed by spraying lightly ninhydrin solution in alcohol 3 g/100 ml, and dried for 15 minutes at 110°C. The retention factor (Rf) value of sample spot obtained should correspond to the standard (USP40-NF35, 2017a).

2.11.2 Uniformity of weight for solid dosage units

Uniformity of weight serves as an indicator of the consistency of active pharmaceutical ingredient in solid dosage units. Weight uniformity test is consequently an important parameter to confirm that the drug content in each drug unit is distributed within a narrow range around the label claim. In oral dosage forms any weight deviation reflects a variation in the content of the active pharmaceutical ingredient (U.S Pharmacopoeia-National Formulary, 2017).

High inconsistencies may alter the dose in each individual drug and cause toxicity or insufficient therapeutic drug levels. Uniformity of weight is an in-process quality parameter that indicates consistency of dosage units during manufacturing (Obarisiagbon et al., 2015; Zaid et al., 2013). Minimum variation between tablets and capsules with respect to dose and weight is a sign of good manufacturing practice (Qureshi, 2016). illustrations the limits of tablet weight variation as stated by the USP and BP (BP, 2017; USP40-NF35, 2017b).

2.11.3 Dissolution test for solid dosage units

Dissolution testing is a standardized method for measuring the rate of drug release from a dosage form. It’s a measurement of the proportion of drug dissolving in a stated time under standardized
conditions (Kraemer et al., 2012). Dissolution testing provides a practical and economic approach to ensure bioequivalence and a good prediction of in-vivo bioavailability of most oral drugs whose inadequacies can mean treatment is ineffective or toxic. The effectiveness of oral dosage forms relies on the drug dissolving in the gastrointestinal fluids prior to absorption (Mann et al., 2017); hence the rate of dissolution is directly correlated to the efficacy of the product (Garbacz and Klein, 2012). Under optimal operating conditions dissolution tests output distinguishes between poor and good drug formulations (Mann et al., 2017).

The BP 2017 specifies use of the basket and paddle apparatus in a suitable dissolution medium at 37± 0.5°C (BP, 2017). The USP 2017 specifies use of basket (USP apparatus 1) for single molecule Amoxicillin 250 mg capsules and Paddle (USP apparatus 2) for 500 mg capsules, employing UV/Vis spectroscopy at a wavelength of 272 nm, 100 and 75 rpm respectively and the dissolution medium at 37± 0.5°C. Amoxicillin/clavulanic acid combination it specifies the use of paddle (USP apparatus 2), detection using LC method, mobile phase methanol and monobasic sodium phosphate buffer adjusted to pH 4.4± 0.5°C using phosphoric acid (1:19), at a wavelength of 220 nm, 4 mm × 30 cm, 3-10 µm packing (C-18) column, 75 rpm and dissolution medium at 37± 0.5°C (USP40-NF35, 2017a).

2.11.4 Potency determination of active pharmaceutical ingredient

Assay tests determines the presence and amounts of active pharmaceutical ingredient(s). A quantity of drug units is selected at random and assay procedures undertaken; the results obtained should be within stated percentage limits. Distinct variations means ineffective therapeutic drug levels or overdosing which results in toxicity (U.S Pharmacopoeia-National Formulary, 2017).
The USP 2017 specifies a LC method with a mobile phase consisting of a mixture of methanol and monobasic sodium phosphate buffer adjusted to pH 4.4± 0.5°C using phosphoric acid (1:19), at a detection wavelength of 220 nm, 4 mm × 30 cm, 3-10 µm packing (C-18) column, with a flow rate of 2 ml/min (USP40-NF35, 2017a).

The BP 2017 specifies the use of LC method with a mixture of acetonitrile and 25.0% v/v solution of 0.2 M potassium hydrogen orthophosphate adjusted to pH 5.0± 0.5°C with 2 M sodium hydroxide (20:80) as the mobile phase with a flow rate of 1 ml/min, 25 cm × 4.6 mm, 5 µm packing with Octadecylsilyl silica gel for chromatography (Hypersil 5 ODS) stainless steel column. Ambient temperature maintained and a detection wavelength of 254 nm and sample injection volume of 50 µl (BP, 2017).

Assay of amoxicillin and its combination amoxicillin/clavulanic acid in dosage forms, animal feed and blood plasma have previously been achieved by several other analytical techniques such as Liquid chromatographic tandem mass spectrometric (LC-MS/MS) (Bhandarkar et al., 2015; Chandra et al., 2012; De Baere and De Backer, 2007; Gaikwad et al., 2013), and infrared Spectroscopy; based on transfectance near infrared (NIR) measurements and partial least squares regression (PLSR) multivariate calibration (Bittner et al., 2011; Lê et al., 2017). This method is simple, more reproducible hence accurate; inexpensive as no solvents required and rapid; eight times faster than LC (Silva et al., 2012). The main disadvantage is its reliance on reference methods and model development using chemo metrics (Manley, 2014).
UV-VIS spectrophotometric method has been used for the assay of amoxicillin two components mixtures (Patel et al., 2013) and pH stimuli sensitive formulation (Tripathi et al., 2014). This method is simple; no sample pretreatment, rapid, and inexpensive and does not need any expensive instrument such as LC and IR spectroscopy (Al-Uzri, 2012). Main disadvantage is lack of sensitivity; samples should be in solution and mixtures are best analyzed after with prior separation (Attia et al., 2016).

Amoxicillin is also assayed by use of capillary electrophoresis (CE). A simple and time efficient method based on capillary zone electrophoresis, was developed for analysis of penicillin derivatives (Cruz Oliva et al., 2011; Tian et al., 2011b; Brigitta and Gyéresi, 2012). Capillary electrophoresis (CE) advantages include small quantity of reagents required, short running time and the ability to analyze unprocessed clinical and environmental samples (Hancu et al., 2014). Disadvantages include; low limits of detection, especially in UV absorbance due to low optical path length, usually used in CE detection technique. Therefore needs high concentration samples or modification of the capillary (Zalewska et al., 2013).

Nuclear magnetic resonance has also been applied for assay of amoxicillin drugs (Aboagye, 2016; USAID, 2018). This method uses complex instrumentation and is highly uneconomical (Baranova et al., 2015).
CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling area

This study was undertaken in Nairobi County, the capital city of Kenya; it hosts the largest airport in East Africa serving as the gateway for imports and exports thus the commercial hub of the country. In addition it is the headquarters of most of the pharmaceutical distributors and wholesalers in the country (Simonetti *et al.*, 2016; Ewen and Okemo, 2018). Figure 3.1 displays Nairobi County map with the study Wards indicated (Kamunya, 2018).

Figure 3.1: Nairobi County map showing the location of the selected Wards (Kamunya, 2018).
Nairobi has Seventeen Sub-counties which are further divided into Eighty-five wards, it has a population of about 3 million people contributing to 8.1% of the Kenyan population hence the most populous county in the Country (Government Of Kenya, 2009).

3.2 Study design

This was a cross-sectional study to identify brands and quality of amoxicillin formulations from retail and private hospital pharmacies different locations in the Nairobi County. A retail pharmacy is any pharmacy business that directs marketing efforts to the final consumer for the purpose of selling goods or services (Simonetti et al., 2016). The locations selection was based on economic stratification; upper, mid and lower income classes (World Bank Group, 2006; Ngugi and Kamula, 2017). The wards with upper income population included Westlands (WST) / Kangemi (KAN) and Karen (KRN), middle income population wards were South B (SB) and South C (SC); middle-lower income populations included Zimmerman (ZIM) and Kasarani (KAS), while lower income population wards were Kibra (KBR), Kayole (KAY) and Umoja (UMJ). Ethical approval was obtained from the Kenya Medical Research Institute- Scientific and Ethics Review Committee (SERU) -KEMRI/SERU/CTMDR/012/3059 (Appendix 1).

A market surveillance was first undertaken in the 10 wards in Nairobi County to identify the various brands, of amoxicillin and amoxicillin/clavulanic acid drugs in July and August of 2016. Additional information such as manufacturer and country of origin were also captured. This was achieved by administering a questionnaire (Appendix 2) where in all the pharmacies sampled, amoxicillin and amoxicillin/clavulanic acid stocked were documented. Pharmacists / Pharmacy attendants in charge of the facility were informed of the nature of the study and assured of
confidentiality (*Appendix 3*), before collecting data or sampling the drugs. Samples were assayed at Kenya Medical Research Institute (KEMRI); Centre for Traditional Medicine and Drug Research laboratories and dissolution studies undertaken at National Quality Control Laboratories (NQCL).

### 3.3 Study sample

The Pharmacy and poisons board (PPB), provided as information that there were 980 pharmacies registered in Nairobi County as of December, 2015. This information did not provide details of location of the pharmacies but informed our sample size; hence the sampling method used was purposive random sampling. The number of retail and private hospital pharmacies sampled from the 10 wards was 278 for the determination of details of brands, their manufacturers and their countries of origin. Subsequently samples for analysis were purchased from 168 retail and hospital pharmacies in the 10 wards. The number of pharmacies were determined based on the Krejie and Morgan’s sample size determination table (Morgan and Krejcie, 1970). The Krejie and Morgan’s sample size calculation was based on \( p=0.05 \), which is expressed as equation (3.1).

\[
S = \frac{X^2 N P(1 - P)}{d^2 (N - 1) + X^2 P(1 - P)}
\]  

(3.1)

Where

- \( S \) = required sample size
- \( X^2 \) = the table value of chi-square for 1 degree of freedom at the desired confidence level (0.05 = 3.841).
- \( N \) = the population size
- \( P \) = the population (assume to be 0.50 since this would provide the maximum sample size.
- \( d \) = the degree of accuracy expressed as proportion (0.05).
In order to eliminate bias, all the amoxicillin and amoxicillin clavulanic acid formulations stocked in each sampled pharmacies were purchased. Two bottles were purchased for oral dry suspensions and at least 50 single units for solid dosages (Tablets and capsules). A total of 148 samples were purchased between September and November 2016 and this formed the primary samples. Thereafter, secondary sampling was carried out purposively by eliminating brands with similar batch numbers, after which systematic sampling was done to achieve a sample size of approximately 50. A random starting point and a fixed periodic interval was selected as shown equation 3.2 (Mostafa and Ahmad, 2018).

\[
\text{Periodic Interval} = \frac{\text{initial sample size}}{\text{Target Sample size}} = \frac{148}{50} = 2.96 \text{ approx. 3} \tag{3.2}
\]

Unique brands were given priority to achieve a sample size of 53, which were analyzed. These consisted of solid dosages; 6 amoxicillin/clavulanic acid tablets of strengths 375 mg and 625 mg and 22 amoxicillin capsules of strengths 250 mg and 500 mg. Oral dry suspensions; 13 amoxicillin oral dry suspension of strengths 125 mg/5 ml and 250 mg/5 ml and 12 amoxicillin/clavulanic acid dry oral suspensions of strengths 228 mg/5 ml and 457 mg/5 ml. Two controls of the innovator drugs; amoxicillin/clavulanic acid tablet of strength 625 mg and amoxicillin oral dry suspension of strength 250 mg/5 ml were sourced from an official local distributer. The samples were assigned codes based on study location and stored in KEMRI pharmaceutical laboratory at room temperature (25°C).

3.4 Chemicals and Reagents

Amoxicillin trihydrate standard (w/w 99.95%) and clavulanate potassium standard (92.75%) from Medopharm private limited (Guduvanchery, India) were generous gifts from Dawa
pharmaceuticals limited, Kenya and National Quality Control Board (NQCL), respectively. Potassium dihydrogen phosphate (Aldrich Chemical Co. Ltd, Gillingham-Dorset, U.K) and phosphoric acid (Merck Pvt. Ltd. Guateng, South Africa) were of analytical grade. Acetonitrile (Sigma-Aldrich Co. Steinheim, Germany) and methanol (Fischer Scientific U.K. Ltd, Loughborough U.K) were of HPLC grade solvents.

3.5 Equipment

The liquid chromatographic system consisted of an Agilent 1260 Infinity series high performance liquid chromatography (LC) system (Agilent Technologies, Deutschland, Germany) supported by Open-Lab software version A.01.03. Equipped with a G1314B Agilent 1260 Infinity variable diode array detector with wavelength range of 190-950 nm with dual lamp design. The temperature was controlled using a G1316A column oven with a G1330B Agilent 1260 Infinity thermostatted column compartment. The LC system also had a pump A G1311C Agilent 1260 Infinity quaternary pump and a G1329B auto sampler.

A dissolution tester (Lab India DS 800, Pvt. Ltd, Mumbai, India) was used. A double beam T90 + UV/VIS spectrophotometer supported by the UVWIN software version 5.2.0 (PG Instruments, Leicestershire, United Kingdom) and quartz cuvettes of a path length of 1 cm were used. A Barnstead Smart2Pure™ water purification system (Thermo Fisher Scientific, Massachusetts, United states) were used to obtain double distilled water.

A Shimadzu AUW220D semi-micro analytical electronic weighing balance (Shimadzu Corporation, Kyoto, Japan) with a sensitivity of ± 0.1 mg was used for weighing. All the mobile
phase preparations were degassed using a DC-200H MRC Ultrasonic cleaner (MRC Lab Ltd, Holon, Israel).

3.6 Procedure for uniformity of weight for solid dosage unit samples
Uniformity of weight determination was only done for single dose preparations (tablets and capsules samples) to check for consistency in weight of dosage units (U.S.P-NF, 2017). This test was undertaken for 28 samples (22 capsules and 6 tablets) plus an innovator product as the control. Twenty units of capsules of strengths 250 mg and 500 mg and tablets of strengths 375 mg and 625 mg from each of the capsules and tablets samples and innovator product (control) of strength 625 mg were taken at random and weighed. The capsules were opened, the contents emptied as completely as possible and the emptied shells were weighed. The net difference was determined, by subtracting the weight of the shells from weight of intact capsule. The weighed tablet units from the respective samples were separately crushed and mixed, similarly the capsule contents were mixed and stored in air tight amber containers and labelled. Average weights of the tablets and capsules for each sample and percentage deviation from the mean value were calculated.

3.7 Procedure for system suitability test (SST)
This test was performed routinely before any sample analyses commenced to verify the proficiency of the chromatographic system. Six replicates injections of the weighed standard were done before any analysis. Content assay of the active ingredients, dissolution testing and impurity determinations must pass a set of predefined acceptance criteria (Table 4.5) before sample analysis can commence (U.S.P-N F, 2017).
3.8 Content determination test

The determination of amoxicillin and clavulanic content was performed using the LC according to criteria established by the USP (USP40-NF35, 2017a).

3.8.1 Mobile phase system preparation

The mobile phase for analysis of amoxicillin capsules and oral dry suspensions consisted of acetonitrile and potassium dihydrogen phosphate buffer pH 5.0 (1:24, v/v). The buffer was prepared by dissolving 6.8 g of monobasic potassium phosphate salt into 1L of double distilled water. The pH was adjusted using potassium hydroxide solution (45% w/w). The mobile phase for analysis of amoxicillin/clavulanic acid tablets and oral suspensions consisted of acetonitrile and potassium dihydrogen phosphate buffer pH 5.0 (1:19, v/v). The buffer solution was prepared by dissolving 7.8 g of monobasic potassium phosphate salt into 1L of double distilled water. The pH was adjusted using phosphoric acid. The buffers were filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filters before use.

3.8.2 Amoxicillin capsules sample preparation

The homogenized contents of each sample stored in air tight amber containers were weighed. A quantity of approximately the average weight obtained from uniformity of weight test done earlier was weighed accurately in triplicates for each sample. Only samples that complied with the compendia specification for uniformity of weight were subjected to API determination. The samples were of strengths 250 mg or 500 mg. Each sample was weighed into 200 ml volumetric flasks and topped to mark with the potassium dihydrogen phosphate buffer. The solutions were sonicated for 10 minutes. Aliquots of 10 ml for 250 mg and 20 ml for 500 mg of the resulting solutions were pipetted in to 25 ml volumetric flasks and topped up to mark with the potassium
dihydrogen phosphate buffer; obtaining the concentration of approximately 1 mg/ ml as per the method adopted (USP40-NF35, 2017a). The solution was filtered through a 0.45 μm syringe adaptable membrane filter into 1.5 ml amber vials and analyzed. Each of the replicates was injected into the LC thrice to obtain the chromatograms.

3.8.3 Amoxicillin dry oral suspensions sample preparation
Total of 13 samples of strengths 125 mg/5 ml and 250 mg/5 ml and innovator product (control) of strength 250 mg/5 ml were reconstituted with distilled water to mark as specified on the labeling; thoroughly mixed and freed of air bubbles. Aliquots of 8 ml (125 mg/5 ml) and 4 ml (250 mg/5 ml) were accurately measured in triplicates using analytical volumetric pipettes into 200 ml volumetric flasks and topped up to mark with the mobile phase. The solutions were sonicated for 10 minutes; to obtain a concentration of approximately 1 mg/ ml. The solutions were filtered using a 0.45 μm membrane filter into 1.5 ml amber vials and analyzed. The samples and innovator product (control) were analyzed on the first (day zero) and on the seventh day after reconstitution to determine their stability. Each of the replicates was injected into the LC thrice.

3.8.4 Amoxicillin standard preparation
As per the compendia (USP 40-NF35, 2017) approximately 12 mg of amoxicillin trihydrate standard potency of 99.95% was accurately weighed in two replicates A and B into a 10 ml volumetric flask, dissolved and made to volume with the buffer solution, obtaining a concentration of about 1.2 mg/ ml. The solution was sonicated for 7 minutes and filtered through a 0.45 μm membrane filters into 1.5 ml amber vials and analyzed. Each of the replicates was injected thrice into the LC to obtain the chromatograms.
3.8.5 Amoxicillin and clavulanic acid tablets sample preparation

Twenty tablets of each of the 6 samples of tablets and the innovator product were separately crushed into fine powder with a pestle and mortar. The weight equivalent to the average weight for each sample were accurately weighed as per the method USP 40-NF 35, (2017).

The tablets were of 375 mg and 625 mg strengths and innovator product (control) of strength 625 mg as per the label claim. Each sample and innovator product were weighed in three replicates into 100 ml volumetric flasks and topped to mark with the potassium dihydrogen phosphate buffer. The solutions were sonicated for 10 minutes. Aliquots of 7 ml (375 mg) and 4 ml (625 mg) of the resulting solutions were pipetted into 50 ml volumetric flasks and made to mark with the potassium dihydrogen phosphate buffer; obtaining the concentration of approximately 0.5 mg/ml. The solutions were filtered through a 0.45 μm membrane filter into 1.5 ml amber vials and analyzed. Each of the replicates was injected thrice into the LC to obtain the chromatograms.

3.8.6 Amoxicillin/clavulanic acid dry oral suspensions sample preparation

Twelve (12) samples of amoxicillin/clavulanic acid dry oral suspension samples (strengths 228.5 mg/5 ml; 457 mg/5 ml and 642.9 mg/5 ml) were all reconstituted with ultrapure water to the mark as specified in the labeling; thoroughly mixed and freed of air bubbles. Triplicate volumes of 4 ml for sample of strength 642.9 mg/5 ml were then taken using analytical volumetric pipettes into 200 ml volumetric flasks and topped up with potassium dihydrogen phosphate buffer. The solution was sonicated for 10 minutes. This made a stock solution from which aliquots of 4 ml was transferred into 20 ml volumetric flask; topped up with potassium dihydrogen phosphate buffer to make the final concentration of approximately 0.5 mg/ml. Same
procedure was repeated for samples of strengths 228.5 mg/5 ml and 457 mg/5 ml where triplicate aliquots of 3 ml were transferred into 100 ml and 200 ml volumetric flasks respectively. This made the stock from which 4 ml was taken into 10 ml volumetric flask to make the final concentration of about 0.5 mg/ ml. The samples were analyzed immediately after reconstituted (day 0) and seven days after reconstitution (day 7) to determine their stability as indicated in the method USP 40-NF 35, (2017). The solution was filtered using a 0.45 μm membrane filter into 1.5 ml amber vials and each replicate was injected thrice into the LC to obtain the chromatograms.

3.8.7 Amoxicillin and clavulanic acid standard preparation
As per the monograph (USP40-NF35,2017) approximately 25 mg of amoxicillin trihydrate standard potency of 99.95% and 10 mg of clavulanate Lithium potency of 96.4% were weighed (mixed) in two replicates A and B to a 50 ml volumetric flask. Dissolved in ultrapure water and topped up to the 50 ml mark to give a final concentration of approximately 0.5 mg/ ml of amoxicillin and 0.2 mg/ml of clavulanate lithium. The solution was filtered through a 0.45 μm membrane filter into 1.5 ml amber vials and each replicate was injected thrice into the LC to obtain the chromatograms.

3.8.8 Chromatographic parameters for potency determination
The wavelength of detection was set at 230 nm and separation was achieved from Symmetry® C18, 4.6 μm 250 × 4 mm column (Waters Corp., Massachusetts, U.S.A) maintained at 40 ± 1°C in a thermostat oven. The injection volume was set at 10μl and the flow rate maintained at 1.5 ml/ min. The samples were the analyzed under these conditions.
3.9 Procedure for Dissolution Studies

Dissolution studies were undertaken for single dose preparations; tablets according to protocol established by the USP (USP40-NF35, 2017a). Dissolution station apparatus 2 and apparatus 1 were set to a frequency of 75 rotations per minute (rpm) for 500 mg capsule samples and 100 rotations per minute (rpm) for 250 mg capsule samples respectively. A set volume of 900 ml of distilled water was poured into each of the six glass vessels and temperature was maintained at 37°C ± 0.5°C for a time duration of 60 minutes. Standard thermometers were placed in each vessel to crosscheck the temperature. Dissolution parameters were set at a frequency of 75 rotations per minute (rpm) and temperature was maintained at 37°C ± 0.5°C for a duration of 30 minutes for the innovator product and tablet samples.

Only samples that complied with the standard requirement for API were subjected to the dissolution studies. These were 19 samples of amoxicillin capsules, 4 samples of amoxicillin/clavulanic acid tablets and the innovator product. Six (6) units of same samples were placed into the dissolution medium (distilled water) all at the same time. This was repeated for each the samples and the innovator product. At the end of the run time, 20 ml were taken from each vessel into a labeled beaker with a calibrated syringe for further analysis. The drug solutions were allowed to equilibrate to room temperature and portions filtered. Amoxicillin and clavulanic acid tablets were transferred into amber vials and analyzed using LC. Triplicate injections were made per sample. Amoxicillin capsules of strengths 250 mg and 500 mg; 10 ml and 5 ml aliquots were respectively transferred into 25 ml volumetric flask. The capsule samples were then analyzed using UV/VIS spectroscopy at a wavelength of 272 nm.
3.9.1 Chromatographic parameters for potency determination and dissolution studies for Amoxicillin/Clavulanic acid tablets

The chromatograph wavelength was set at 220 nm and separation was achieved from Symmetry® C18 4.6 μm 25 cm × 4 mm column (Waters Corp., Massachusetts, U.S.A) maintained at 40 ± 1°C in a thermostat oven. The injection volumes were set at 20μl and the flow rate was maintained at 2.0 ml/ min. The column temperature was maintained at 25 ± 1°C in a thermostat oven. The samples were analyzed under these conditions.

3.10 Statistical analysis

The data obtained was given as the mean values ± SD of at least three independent experiments with at least triplicates for each by using Excel MS 2010 and Statistical Package for Social Scientists (SPSS) version 21.0 software.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Frequency, location and countries of origin of amoxicillin and amoxicillin/clavulanic acid products

Ten wards in Nairobi County were purposively sampled to represent different socio-economic settings; upper, middle- and lower-income classes (Ngugi and Kamula, 2017). Out of 278 pharmacies visited, 99.6% (277) pharmacies stocked amoxicillin formulations. Innovator brands for both single molecule amoxicillin formulations (Amoxil®) and amoxicillin/clavulanic acid combination (Augmentin®) were stocked in 72.3% (201 out of 278) of pharmacies visited (Table 4.1).

Table 4.1: Summary on pharmacies that stocked amoxicillin formulations in Nairobi County.

<table>
<thead>
<tr>
<th>Nairobi County</th>
<th>Number of Pharmacies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacies sampled</td>
<td>278</td>
</tr>
<tr>
<td>Stocking Amoxicillin</td>
<td>277 (99.6%)</td>
</tr>
<tr>
<td>Stocking Amoxicillin Innovator Brands</td>
<td>201 (72.3%)</td>
</tr>
<tr>
<td>Not stocking Amoxicillin innovator Brands</td>
<td>77 (28%)</td>
</tr>
<tr>
<td>Stocking Amoxicillin generic Brands</td>
<td>264 (95%)</td>
</tr>
<tr>
<td>Not stocking Amoxicillin generic Brands</td>
<td>14 (5.0%)</td>
</tr>
</tbody>
</table>

This concurs with the WHO Expert Committee, (2017) report on essential medicines that recommends amoxicillin as a drug with lower potential for resistance and should be readily accessible for treatment of a wide range of common bacterial infections. A total of 106 different brands were found in the market, 49.0% of which were single molecule amoxicillin imports and
31.1% were amoxicillin/clavulanic acid combinations imports. Locally manufactured brands accounted for 19.8% of the brands recorded in Nairobi County and were single molecule amoxicillin (Figure 4.1).

![Diagram showing the frequency of brands of amoxicillin products in Nairobi County](chart)

**Figure 4.1:** Frequency of brands of amoxicillin products in Nairobi County

Amoxil® accounted for 19.3% of all amoxicillin products recorded in the selected locations while Augmentin® was 15.9% as shown in **Figure 4.2 A and B** respectively. Commonly stocked generics for single molecule formulations were Penamox® (7.7%) and amoxicillin/ clavulanic acid combination was Clavulin® (8.5%) as indicated in **Figure 4.2**.
Figure 4.2: Predominant brands of (A) amoxicillin and (B) amoxicillin/clavulanic acid drugs stocked in sampled pharmacies within Nairobi County.
Innovator brands Amoxil® and Augmentin® were predominantly stocked in Nairobi Central (CBD) (28.2%) a location that serves all income classes and in high- and mid-income areas; 14.4% being in Westlands, 10.4% in Karen, 7.7% in South C and 9.3% in South B as compared to low and middle lower income areas where 4.5% were stocked in Kibra, 6.2% in Kayole, 6.0% in Zimmerman and 7.2% in Kasarani (Table 4.2).

Table 4.2: Percentage frequency of Innovator brands (Amoxil® and Augmentin®) in the sampled locations

<table>
<thead>
<tr>
<th>Location</th>
<th>Socio-Economical Class</th>
<th>Percentage Frequency of Innovator (Amoxil® and Augmentin®) brands (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nairobi Central (CBD)</td>
<td>Cuts across all income classes</td>
<td>28.2</td>
</tr>
<tr>
<td>Karen (KRN)</td>
<td>Upper Income</td>
<td>10.4</td>
</tr>
<tr>
<td>Westlands/ Kangemi (WST/KAN)</td>
<td></td>
<td>14.4</td>
</tr>
<tr>
<td>South C (SC)</td>
<td>Middle income</td>
<td>7.7</td>
</tr>
<tr>
<td>South B (SB)</td>
<td></td>
<td>9.3</td>
</tr>
<tr>
<td>Kasarani (KAS)</td>
<td>Middle –lower income</td>
<td>7.2</td>
</tr>
<tr>
<td>Zimmerman (ZIM)</td>
<td></td>
<td>6.6</td>
</tr>
<tr>
<td>Umoja (UM)</td>
<td>Lower Income</td>
<td>5.6</td>
</tr>
<tr>
<td>Kayole (KAY)</td>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td>Kibra (KBR)</td>
<td></td>
<td>4.5</td>
</tr>
</tbody>
</table>
Similar findings were reported in a survey on the medicine pricing in Kenya which noted that innovator brands were mostly sold in Nairobi with their availability being twice more likely than in other regions in Kenya (HAI and WHO, 2004).

Generally, Nairobi central (CBD) had the highest frequency of amoxicillin drugs stocked in pharmacies at 25.7% as shown in Figure 4.3, followed by Westlands at 13.5%. CBD and Westlands wards hosts most of the distributer outlets from which pharmacies within Nairobi County source their stock (Ministry of Health, 2016). Pharmacies in South C area had the lowest number of amoxicillin products stocked at 3.6%. This was expected since South C is a middle-income class residential estate, with a population of approximately 50,000 hence fewer pharmacies located in the ward (Ngugi and Kamula, 2017).

Figure 4.3. Frequency of amoxicillin and amoxicillin/ clavulanic acid brands available in sampled wards within Nairobi County
Additionally, 85.2% of amoxicillin products were imports with 44.0% being generic products, while 41.3% were innovator imported products (Amoxil® and Augmentin®). 25.6% of the innovator brands were from the United Kingdom, 8.6% from France and 6.9% from Belgium (Figure 4.4.A). Majority of the generic amoxicillin brands were from India (27.4%), Canada (6.4%) and China (2.8%) as shown in Figure 4.4 B and C.

**Figure 4.4:** Frequency of common brands of amoxicillin and amoxicillin/Clavulanic acid based on continent of origin (A) Asia (B) Europe (C) Africa and (D) America.
This concurred with findings from a report on prices and availability of medicines in the Kenyan market that demonstrated the majority of imported generics are manufactured in India followed by China (Ewen and Okemo, 2018). Additionally, noted was that all the combination amoxicillin/clavulanic acid drugs found in Nairobi market were imports.

### 4.2 Uniformity of weight for solid unit dosages in Capsules and Tablets

A total of 28 samples and a control (innovator product) of single dose preparations were subjected to uniformity of weight test and percentage weight variation was compared to the limits defined by USP official monograph (U.S. Pharmacopoeia-National Formulary, 2017b). From Table 4.3, the innovator product and all amoxicillin/clavulanic acid tablets sampled were within the acceptable percentage mean weight deviation of ± 5.0% for uniformity of weight for tablets with API above 324 mg as per the USP recommended limits.

**Table 4.3:** Uniformity of weight data for amoxicillin/clavulanic acid tablets sampled from various pharmacies in Nairobi County.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Country of origin</th>
<th>Labelled active ingredients (mg)</th>
<th>Average weight uniformity (mg) ± SD</th>
<th>Mean weight deviation%</th>
<th>Number of tablets outside USP range</th>
<th>USP Acceptable Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innovator</td>
<td>United Kingdom</td>
<td>625</td>
<td>1083.9 ± 9.11</td>
<td>0.8</td>
<td>-1.7300–1.2800</td>
<td>±5%</td>
</tr>
<tr>
<td>KAN95</td>
<td>India</td>
<td>375</td>
<td>739.7 ± 9.07</td>
<td>1.2</td>
<td>-1.9880–2.7166</td>
<td>±5%</td>
</tr>
<tr>
<td>SB36</td>
<td>India</td>
<td>375</td>
<td>784.6 ± 6.69</td>
<td>0.9</td>
<td>-1.7334–1.2236</td>
<td>±5%</td>
</tr>
<tr>
<td>CBD131</td>
<td>India</td>
<td>625</td>
<td>1091.0 ± 14.84</td>
<td>1.4</td>
<td>-2.9532–2.1429</td>
<td>±5%</td>
</tr>
<tr>
<td>KBR90</td>
<td>India</td>
<td>625</td>
<td>1023.1 ± 17.89</td>
<td>1.7</td>
<td>-3.0920–2.8506</td>
<td>±5%</td>
</tr>
<tr>
<td>SB57</td>
<td>India</td>
<td>625</td>
<td>1041.0 ± 12.10</td>
<td>1.2</td>
<td>-3.1586–1.9424</td>
<td>±5%</td>
</tr>
<tr>
<td>CBD136</td>
<td>India</td>
<td>625</td>
<td>1051.1 ± 11.34</td>
<td>1.1</td>
<td>-2.2771–1.6425</td>
<td>±5%</td>
</tr>
</tbody>
</table>

RSD- Relative Standard Deviation, SD- Standard deviation.
These data agree with a study done in Ethiopia, on amoxicillin/clavulanic tablets, where all samples analyzed were within the stated standard ranges for weight uniformity (Mekonnen et al., 2016). This uniformity of weight is a pointer of adherence to good manufacturing practice (GMP) when the amount of active pharmaceutical ingredient (API) contained in a formulation is uniform. It assures that drug content in each unit dose is distributed in a narrow range around the label active ingredient assuring consistent and correct dosage when prescribed (Qureshi, 2016). Table 4.4 shows that, 86.4% (19 out of 22) amoxicillin capsule samples complied with the USP compendia specifications for uniformity of weight while 13.6% (3 out of 22) failed to meet the USP stated limits.
Table 4.4: Uniformity of weight results of amoxicillin capsules sampled from various pharmacies in Nairobi County.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Drug origin</th>
<th>Labelled active ingredient(s) (mg)</th>
<th>Average weight uniformity (mg)±SD</th>
<th>Mean weight deviation %</th>
<th>Number of capsules outside USP range</th>
<th>USP Acceptable Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAY52</td>
<td>China</td>
<td>250</td>
<td>312.8± 5.31</td>
<td>1.7</td>
<td>-1.8862~3.1969</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>SC10</td>
<td>Kenya</td>
<td>250</td>
<td>288.7 ± 8.37</td>
<td>2.9</td>
<td>-5.2650~4.8147</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>SB32</td>
<td>India</td>
<td>250</td>
<td>301.9± 6.07</td>
<td>2.0</td>
<td>-4.0742~5.2004</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>KAY56</td>
<td>India</td>
<td>500</td>
<td>594.8± 5.83</td>
<td>1.0</td>
<td>-2.1520~1.9670</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KAY51</td>
<td>India</td>
<td>250</td>
<td>309.0± 9.96</td>
<td>3.2</td>
<td>-8.2201~6.9579</td>
<td>1 ±7.5%</td>
</tr>
<tr>
<td>ZIM109</td>
<td>Kenya</td>
<td>250</td>
<td>295.7± 5.59</td>
<td>1.9</td>
<td>-3.5171~4.1258</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>CBD138</td>
<td>Kenya</td>
<td>250</td>
<td>336.6±10.84</td>
<td>3.2</td>
<td>-5.7932~4.8128</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>KBR78</td>
<td>China</td>
<td>250</td>
<td>300.0± 7.70</td>
<td>2.6</td>
<td>-3.2000~5.000</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>KBR84</td>
<td>Kenya</td>
<td>500</td>
<td>591.7± 4.49</td>
<td>0.8</td>
<td>-2.0788~1.1323</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KBR87</td>
<td>India</td>
<td>250</td>
<td>296.9± 6.47</td>
<td>2.2</td>
<td>-4.8501~2.8629</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>KBR67</td>
<td>Kenya</td>
<td>250</td>
<td>436.5± 9.91</td>
<td>2.3</td>
<td>-5.0401~3.3219</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>KRN94</td>
<td>Mexico</td>
<td>500</td>
<td>587.2 ± 7.38</td>
<td>1.3</td>
<td>-0.0249~0.0189</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KBR77</td>
<td>Kenya</td>
<td>500</td>
<td>592.5± 8.30</td>
<td>1.4</td>
<td>-2.3122~2.4810</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KAN120</td>
<td>United Kingdom</td>
<td>500</td>
<td>588.6± 6.58</td>
<td>1.1</td>
<td>-2.3955~1.9198</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>SC13</td>
<td>India</td>
<td>500</td>
<td>579.9± 7.75</td>
<td>1.33</td>
<td>-2.5349~2.2935</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>SB21</td>
<td>China</td>
<td>500</td>
<td>585.4± 11.17</td>
<td>1.9</td>
<td>-2.6478~4.0656</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KBR84</td>
<td>Kenya</td>
<td>500</td>
<td>591.7± 4.49</td>
<td>0.8</td>
<td>-2.0788~1.1323</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KBR87</td>
<td>India</td>
<td>250</td>
<td>296.9± 6.47</td>
<td>2.2</td>
<td>-4.8501~2.8629</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>KBR67</td>
<td>Kenya</td>
<td>250</td>
<td>436.5± 9.91</td>
<td>2.3</td>
<td>-5.0401~3.3219</td>
<td>0 ±7.5%</td>
</tr>
<tr>
<td>KRN94</td>
<td>Mexico</td>
<td>500</td>
<td>587.2 ± 7.38</td>
<td>1.3</td>
<td>-0.0249~0.0189</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KBR77</td>
<td>Kenya</td>
<td>500</td>
<td>592.5± 8.30</td>
<td>1.4</td>
<td>-2.3122~2.4810</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>CBD128</td>
<td>United Kingdom</td>
<td>500</td>
<td>589.6± 5.93</td>
<td>1.0</td>
<td>-1.3569~1.7639</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KAY42</td>
<td>China</td>
<td>500</td>
<td>593.2 ± 3.77</td>
<td>0.6</td>
<td>-1.0115~1.3823</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>SC12</td>
<td>India</td>
<td>500</td>
<td>589.9± 8.80</td>
<td>1.5</td>
<td>-3.9329~2.9836</td>
<td>0 ±5.0%</td>
</tr>
<tr>
<td>KBR89</td>
<td>India</td>
<td>500</td>
<td>587.8±23.99</td>
<td>4.1</td>
<td>-10.496~4.2361</td>
<td>3 ±5.0%</td>
</tr>
<tr>
<td>CBD127</td>
<td>China</td>
<td>500</td>
<td>587.8± 23.57</td>
<td>4.0</td>
<td>-6.822~9.0167</td>
<td>8 ±5.0%</td>
</tr>
<tr>
<td>KAN101</td>
<td>India</td>
<td>500</td>
<td>547.6±29.57</td>
<td>5.4</td>
<td>-8.6591~7.1950</td>
<td>9 ±5.0%</td>
</tr>
</tbody>
</table>

RSD - Relative Standard Deviation, SD- Standard deviation
The observed weight differences in the failed samples reflect on the content of active ingredient as inconsistent hence if prescribed the patient will not receive the correct dosage and may result in suboptimal dosages or toxicity (Johnston and Holt, 2014; Shabana, 2016). The samples that failed were imports and were eliminated from further tests.

Analysis of solid dosages of capsules and tablets indicated that 89.0% (25 out of 28) samples and the innovator product were within the USP limits. According to the USP, not more than two of the individual masses should deviate from the average mass of the percentage weight deviation, and none should deviate more than twice that percentage weight (U.S. Pharmacopoeia-National Formulary, 2017). Only one-unit capsule of sample KAY51 deviated from the mean beyond the USP specified limit of ± 7.5% for drugs with API of strength 250mg; hence considered within required standard for uniformity of weight. The samples that failed KBR89, KAN101 and CBD127 differed in mass from the average by more than the USP compendium permitted limit of ± 5.0% for 500 mg solid unit dosages (U.S. Pharmacopoeia-National Formulary, 2017b). Sample KBR89 had 3 capsules with masses below the acceptable limit and 1 capsule weight deviating more than double the acceptable limit; CBD127 had 4 capsules with masses below and 4 capsules with masses above the acceptable limits and KAN 101 had 5 capsules with masses below and 4 capsules with masses above the acceptable limits. This agreed with a study carried out in Nigeria on amoxicillin capsules in which 30.0% (3 out of 10) of samples weighed failed to comply with compendia limits (Obarisiagbon et al., 2015). Once such weight variances are established it is thereafter impossible to assure the required API content of the drug units (Kendall et al., 1981; Zhigang et al., 2010).
4.3 System suitability parameters for samples analysis using LC

Liquid chromatography (LC) was used for content determination of the API in all the samples. The chromatographic system parameters were within the USP limits as given in Table 4.5. The chromatographic system suitability test were undertaken before any analysis to ensure the parameters are within pharmacopoeia limits. This test is an integral part of any analytical procedure; verifies resolution, column efficiency and repeatability of a chromatographic system to guarantee its competence for a particular analysis (Kumar Sahu, 2017).

Table 4.5: Method System suitability Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method System Suitability Parameters</th>
<th>Acceptable Criteria (USP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amoxicillin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clavulanic Acid</td>
<td></td>
</tr>
<tr>
<td>Precision/Injection</td>
<td>Indicated in the data for respective samples in the results</td>
<td>Relative Standard Deviation ≤ 2%</td>
</tr>
<tr>
<td>Repeatability</td>
<td>tables.</td>
<td></td>
</tr>
<tr>
<td>Resolution Factor(R)</td>
<td>2.76 to 3.17</td>
<td>R &gt; 1.5</td>
</tr>
<tr>
<td>Tailing Factor(T)</td>
<td>0.89 to 1.52</td>
<td>T ≤ 2</td>
</tr>
<tr>
<td>Theoretical Plates (N)</td>
<td>2017-2470</td>
<td>N ≥ 2000 Plates</td>
</tr>
<tr>
<td></td>
<td>3970-4480</td>
<td></td>
</tr>
</tbody>
</table>

4.4 Active Pharmaceutical Ingredient Confirmatory in samples using LC

Identification test was first undertaken to ascertain that the samples contained the targeted API using LC as per USP method (USP40-NF35, 2017a). The retention times of major peaks of the samples of all amoxicillin and amoxicillin /clavulanic acid brands corresponded to that of the reference standards as shown in chromatograms in Figure 4.5. Single molecule amoxicillin samples (II and III) and the standard (I) retention time was at 2.7 ± 0.2 minutes. In the
combination samples (V and VI) and standard molecule (IV); amoxicillin ranged at $2.5 \pm 0.2$ minutes while clavulanic acid was $4.9 \pm 0.2$ minutes as shown in chromatogram in Figure 4.5. This confirmed that all samples analyzed contained the targeted active pharmaceutical ingredients (Moldoveanu et al., 2013).

**Figure 4.5:** Typical chromatograms from analyzed samples. Chromatographic parameters: C18 4.6 μm 250 × 4 mm column, Temperature 40 °C, injection volume 10μl and flow rate 1.5 ml/min.
4.5 Content Determination using LC

4.5.1 Content determination for Amoxicillin oral dry suspensions

The quantification of API was undertaken using LC as per USP monograph (USP40-NF35, 2017a). A total of 13 amoxicillin oral dry suspensions samples and an innovator product were analyzed and the data given on Table 4.6.

Table 4.6: Percentage of active pharmaceutical ingredient (API) in amoxicillin dry oral suspensions sampled in Nairobi County.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Drug Origin</th>
<th>Labelled active ingredients (mg/5 ml)</th>
<th>Chemical content as % label claim (±RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>Innovator</td>
<td>United Kingdom</td>
<td>250</td>
<td>93.56 (0.26)c</td>
</tr>
<tr>
<td>KAN118</td>
<td>Kenya</td>
<td>125</td>
<td>98.56 (1.36)b</td>
</tr>
<tr>
<td>KBR63</td>
<td>India</td>
<td>125</td>
<td>99.56 (0.25)b</td>
</tr>
<tr>
<td>KBR82</td>
<td>India</td>
<td>125</td>
<td>101.18 (0.96)b</td>
</tr>
<tr>
<td>KAY43</td>
<td>India</td>
<td>125</td>
<td>98.56 (0.68)b</td>
</tr>
<tr>
<td>KBR83</td>
<td>Kenya</td>
<td>125</td>
<td>95.60 (1.12)c</td>
</tr>
<tr>
<td>SC06</td>
<td>Kenya</td>
<td>125</td>
<td>104.21 (0.97)a</td>
</tr>
<tr>
<td>ZIM106</td>
<td>Kenya</td>
<td>125</td>
<td>102.97 (0.77)a</td>
</tr>
<tr>
<td>ZIM113</td>
<td>India</td>
<td>125</td>
<td>101.58 (0.33)b</td>
</tr>
<tr>
<td>KAY54</td>
<td>United Arab Emirates</td>
<td>250</td>
<td>100.22 (0.21)b</td>
</tr>
<tr>
<td>SB38</td>
<td>Kenya</td>
<td>250</td>
<td>103.91 (1.05)a</td>
</tr>
<tr>
<td>SB19</td>
<td>Egypt</td>
<td>125</td>
<td>92.81 (1.00)c</td>
</tr>
<tr>
<td>SC03</td>
<td>India</td>
<td>125</td>
<td>91.30 (1.57)c</td>
</tr>
<tr>
<td>KBR74</td>
<td>India</td>
<td>125</td>
<td>86.02 (0.53)d</td>
</tr>
</tbody>
</table>

RSD-Relative Standard Deviation, USP limits 90%-120% of Label Claim
Percentages (descending) with different superscript letters with respect to the innovator are significantly different (p < 0.05).
It was noted that on day zero, 92.0% (12 out of 13) of amoxicillin oral suspensions samples and the innovator product met the specified USP limits for potency while on day seven, 85.0% (11 out of 13) of the samples and the innovator product were still within the USP limits. According to USP, amoxicillin formulations should contain not less than 90.0% and not more than 120.0% of the stated label claim of the active ingredient all through the prescription period (day 0 to day 7) (USP40-NF35, 2017a). However, 23.1% (3 out of 13) failed to meet the USP limits for assay as presented in **Table 4.6** and all were imported products. Sample SB19 was sampled from South B and manufactured in Egypt, Sample SC03 and KBR74 were both manufactured in India and sampled from South C and Kibra respectively.

Content determination test is critical for quality assessment of API to attain effective concentration at the site of infections when administered (Almuzaini *et al*., 2013; Butler *et al*., 2013). According to WHO, (2018) substantial variations could result in ineffective or toxic therapeutic drug levels. This data is consistent with the findings of Taylor *et al*., (2001) that reported low API quantities in 40.0% (2 out of 5) amoxicillin oral dry suspensions analyzed. Similar conclusions was also documented in Saudi Arabia where on day zero only 8.0% (1 out of 13) amoxicillin suspension samples was below USP limits while on day 38.0 % (7 out of 13) were outside the pharmacopeia limits (Kyriacos *et al*., 2008). A significant difference (p < 0.05) was observed when the chemical content for the amoxicillin oral suspension innovator product and samples were compared.
4.5.2 Content determination for Amoxicillin/Clavulanic acid oral dry suspensions

Twelve amoxicillin/clavulanic acid oral dry suspensions samples and an innovator product as the control were analyzed to determine the active pharmaceutical ingredient and the data given in Table 4.7.

**Table 4.7:** Percentage active pharmaceutical ingredient (API) results for amoxicillin/clavulanic acid dry oral suspensions sampled from various pharmacies in Nairobi County.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Country of Origin</th>
<th>Label API (mg)</th>
<th>Chemical content as % label claim (±RSD) for Clavulanic Acid</th>
<th>Chemical content as % label claim (±RSD) for Amoxicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAN119</td>
<td>India</td>
<td>228</td>
<td>Day 0: 112.73 (1.78) (^a) Day 7: 100.01 (0.84) (^b)</td>
<td>Day 0: 108.23 (0.94) (^c) Day 7: 102.00 (0.20) (^d)</td>
</tr>
<tr>
<td>KBR60</td>
<td>United Arab Emirates</td>
<td>228</td>
<td>113.48 (0.47) (^a) Day 7: 102.20 (0.58) (^a)</td>
<td>Day 0: 104.22 (0.27) (^a) Day 7: 100.04 (0.37) (^b)</td>
</tr>
<tr>
<td>KRN93</td>
<td>Canada</td>
<td>457</td>
<td>98.99 (0.83) (^c) Day 7: 90.62 (0.48) (^c)</td>
<td>Day 0: 102.24 (0.33) (^b) Day 7: 96.20 (0.66) (^b)</td>
</tr>
<tr>
<td>SC05</td>
<td>United Kingdom</td>
<td>228</td>
<td>99.51 (0.93) (^c) Day 7: 96.18 (0.70) (^c)</td>
<td>Day 0: 96.53 (0.68) (^c) Day 7: 90.53 (0.65) (^c)</td>
</tr>
<tr>
<td>WST98</td>
<td>India</td>
<td>228</td>
<td>101.03 (0.23) (^d) Day 7: 96.67 (0.48) (^c)</td>
<td>Day 0: 91.66 (1.83) (^c) Day 7: 86.83 (0.80) (^d)</td>
</tr>
<tr>
<td>KBR80</td>
<td>India</td>
<td>228</td>
<td>105.49 (1.29) (^a) Day 7: 98.34 (0.24) (^c)</td>
<td>Day 0: 92.02 (1.25) (^c) Day 7: 88.85 (1.95) (^d)</td>
</tr>
<tr>
<td>CBD132</td>
<td>India</td>
<td>228</td>
<td>116.93 (0.64) (^a) Day 7: 114.64 (0.73) (^a)</td>
<td>Day 0: 92.54 (1.33) (^c) Day 7: 89.12 (1.97) (^d)</td>
</tr>
<tr>
<td>SC11</td>
<td>France</td>
<td>642.9</td>
<td>96.50 (0.75) (^c) Day 7: 88.91 (0.58) (^d)</td>
<td>Day 0: 96.90 (0.57) (^c) Day 7: 90.75 (1.15) (^c)</td>
</tr>
<tr>
<td>KAY40</td>
<td>Jordan</td>
<td>642.9</td>
<td>94.43 (0.69) (^c) Day 7: 86.35 (0.87) (^d)</td>
<td>Day 0: 94.29 (0.22) (^c) Day 7: 91.75 (0.77) (^c)</td>
</tr>
<tr>
<td>SC08</td>
<td>India</td>
<td>228</td>
<td>101.70 (0.28) (^b) Day 7: 96.61 (0.21) (^c)</td>
<td>Day 0: 82.00 (0.93) (^e) Day 7: 80.22 (0.30) (^e)</td>
</tr>
<tr>
<td>CBD137</td>
<td>India</td>
<td>457</td>
<td>94.03 (1.00) (^c) Day 7: 91.30 (1.77) (^c)</td>
<td>Day 0: 89.76 (0.90) (^d) Day 7: 83.22 (1.03) (^f)</td>
</tr>
<tr>
<td>CBD133</td>
<td>India</td>
<td>228</td>
<td>93.43 (0.8) (^c) Day 7: 89.93 (0.67) (^d)</td>
<td>Day 0: 85.12 (1.63) (^c) Day 7: 82.07 (0.57) (^e)</td>
</tr>
</tbody>
</table>

RSD—Relative Standard Deviation, USP limits: Amoxicillin: 90%-120% and clavulanic acid 90%-125% of label claim. Percentages (descending) with different superscript letters with respect to the innovator on Table 4.7 are significantly different (p < 0.05).
On day zero, 75.0% (9 out of 12) of samples determined for content were within the USP specifications for API while 25.0% (3 out of 12) of the samples that did not meet the USP limits failed for amoxicillin assay content only. According to the pharmacopoeia guidelines amoxicillin/clavulanic acid formulations should contain not less than 90.0% and not more than 120.0% of the stated label claim of the active ingredient for amoxicillin and not less than 90.0% and not more than 125.0% of the labeled amount for clavulanic acid (USP40-NF35, 2017a). Furthermore, a sample has to be within these limits for both amoxicillin and clavulanic acid immediately after reconstitution (day 0) and on seventh day (day 7) in order to be considered as having passed content analysis. On day seven only 33.0% (4 out of 12) of samples were within USP specifications for content assay for both active ingredients. Majority of samples 50.0% (6 out of 12) were below pharmacopoeia limits for amoxicillin content. Nettey, Allotey-Babington, et al., (2014) reported comparable findings in Ghana with amoxicillin content showing more failure than clavulanic acid. In total 66.7% (8 out of 13) were outside the specified limits and were imported products; 6 manufactured in India and remaining 2 from Egypt and Jordan. There was significant difference (p < 0.05) on comparing the chemical content for innovator product and the amoxicillin/clavulanic oral dry suspension samples were compared.

4.5.3 Percentage content for Amoxicillin capsules and amoxicillin/clavulanic acid tablets

Nineteen (19) amoxicillin capsules samples of strengths 250 mg and 500 mg were analyzed, all met the specified USP limits for amoxicillin as indicated in Table 4.8.
Table 4.8: Percentage active pharmaceutical ingredient (API) for amoxicillin capsules sampled from various pharmacies in Nairobi County.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Country of Origin</th>
<th>Labelled active ingredients (mg)</th>
<th>Chemical content as % label claim (±RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAY52</td>
<td>China</td>
<td>250</td>
<td>95.91 (0.71)(^b)</td>
</tr>
<tr>
<td>SC10</td>
<td>Kenya</td>
<td>250</td>
<td>98.24 (0.26)(^b)</td>
</tr>
<tr>
<td>SB032</td>
<td>India</td>
<td>250</td>
<td>93.93 (0.27)(^c)</td>
</tr>
<tr>
<td>KAY56</td>
<td>India</td>
<td>500</td>
<td>97.08 (0.34)(^b)</td>
</tr>
<tr>
<td>KAY51</td>
<td>India</td>
<td>250</td>
<td>98.60 (0.66)(^b)</td>
</tr>
<tr>
<td>ZIM109</td>
<td>Kenya</td>
<td>250</td>
<td>95.98 (0.29)(^b)</td>
</tr>
<tr>
<td>CBD138</td>
<td>Kenya</td>
<td>250</td>
<td>98.12 (1.92)(^b)</td>
</tr>
<tr>
<td>KBR78</td>
<td>China</td>
<td>250</td>
<td>94.09 (1.12)(^c)</td>
</tr>
<tr>
<td>KAN120</td>
<td>United Kingdom</td>
<td>500</td>
<td>96.67 (0.63)(^c)</td>
</tr>
<tr>
<td>SC13</td>
<td>India</td>
<td>500</td>
<td>94.20 (0.95)(^c)</td>
</tr>
<tr>
<td>SB21</td>
<td>China</td>
<td>500</td>
<td>92.60 (1.64)(^c)</td>
</tr>
<tr>
<td>KBR84</td>
<td>Kenya</td>
<td>500</td>
<td>94.34 (1.99)(^c)</td>
</tr>
<tr>
<td>KBR87</td>
<td>India</td>
<td>250</td>
<td>95.06 (0.23)(^b)</td>
</tr>
<tr>
<td>KBR67</td>
<td>Kenya</td>
<td>250</td>
<td>90.74 (0.55)(^c)</td>
</tr>
<tr>
<td>KRN94</td>
<td>Mexico</td>
<td>500</td>
<td>91.94 (0.54)(^c)</td>
</tr>
<tr>
<td>KBR77</td>
<td>Kenya</td>
<td>500</td>
<td>94.78 (0.11)(^c)</td>
</tr>
<tr>
<td>CBD128</td>
<td>United Kingdom</td>
<td>500</td>
<td>96.57 (0.26)(^c)</td>
</tr>
<tr>
<td>KAY42</td>
<td>China</td>
<td>500</td>
<td>94.89 (0.62)(^c)</td>
</tr>
<tr>
<td>SC12</td>
<td>India</td>
<td>500</td>
<td>93.91 (0.46)(^c)</td>
</tr>
</tbody>
</table>

RSD-Relative Standard Deviation, USP limits: content determination ~90%-120% and dissolution ~not less than (NLT) 65% of label claim. Percentages (descending) with different superscript letters with respect to the innovator on Table 4.4 are significantly different (p < 0.05).

Comparable results were reported in Ethiopia, where all 8 amoxicillin capsule samples from pharmacies in Addis Ababa complied with specified limits (Mikre and Mekonnen, 2009).
Similarly, a study conducted in Ghana, Nigeria and United kingdom on amoxicillin formulations concluded that 95.0% (19 out of 20) of amoxicillin capsules analyzed complied to USP tolerance limits (Kaur et al., 2015). On the contrary a study conducted in India on generic amoxicillin capsules purchased from open markets recorded 28.3% (13 out of 46) of the products were out of the IP specifications (Khan et al., 2015). A study by Nguyo et al., (2013) also reported 50.0% (11 out of 22) none-compliance with USP specifications for amoxicillin capsules analyzed in Kenya all of which were of local origin, over a period of 2006-2010. A significant difference (p < 0.05) was observed when the chemical content for the innovator product and amoxicillin capsules and samples were compared. From Table 4.9, 66.7% (4 out of 6) of amoxicillin/clavulanic acid tablets analyzed and the innovator product were within the specifications for API content.
Table 4.9: Percentage active pharmaceutical ingredient (API) results for amoxicillin/clavulanic acid tablets sampled from various pharmacies in Nairobi County.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Country of Origin</th>
<th>Labelled active ingredients (mg)</th>
<th>Chemical content as % label claim (±RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clavulanic Acid</td>
</tr>
<tr>
<td>Innovator</td>
<td>United Kingdom</td>
<td>625</td>
<td>94.00 (0.99)c</td>
</tr>
<tr>
<td>KAN095</td>
<td>India</td>
<td>375</td>
<td>99.82 (0.59)c</td>
</tr>
<tr>
<td>SB036</td>
<td>India</td>
<td>375</td>
<td>110.67 (1.37)a</td>
</tr>
<tr>
<td>CBD131</td>
<td>India</td>
<td>625</td>
<td>100.80 (0.35)b</td>
</tr>
<tr>
<td>KBR090</td>
<td>India</td>
<td>625</td>
<td>94.50 (0.50)c</td>
</tr>
<tr>
<td>SB057</td>
<td>India</td>
<td>625</td>
<td>95.40 (1.19)c</td>
</tr>
<tr>
<td>CBD136</td>
<td>India</td>
<td>625</td>
<td>108.10 (1.28)a</td>
</tr>
</tbody>
</table>

RSD-Relative Standard Deviation, USP limits: content determination for amoxicillin-90% - 120%, clavulanic acid 90% - 125% of label claim. Percentages (descending) with different superscript letters with respect to the innovator are significantly different (p < 0.05).

The samples (KAN 095 and CBD136) that did not meet the specified limits failed for amoxicillin content and were both imported products. They were hence eliminated from any further tests. Contrasting observations were demonstrated in Ghana where all amoxicillin /clavulanic acid tablets analyzed were outside required limits (Nettey et al., 2014). Olanrewaju et al., (2012) and Mekonnen et al., (2016) from Nigeria and Ethiopia respectively reported compliance with compendium limits for all amoxicillin/clavulanic acid tablets samples analyzed. Many factors may affect the content of a drug product such as transportation factors during distribution, storage factors components of drug composition and nature of the active ingredient used during formulation (Kelesidis et al., 2007). It is however notable that the percentage contents were rather closer to the lower limit stated by the USP pharmacopoeia. This could be attributed to
poor manufacturing and or distribution processes (Kelesidis et al., 2007). There was significant difference (p < 0.05) on comparing the chemical content for innovator product and the amoxicillin/clavulanic acid tablet samples were compared.

Generally, 75.5% (40 out of 53) samples analyzed were of good quality as per USP specifications while 24.5% (13 out of 53) failed to meet the limits. A drug whose active pharmaceutical ingredient is below the therapeutic dosage is considered to be of substandard quality (Shabana, 2016). The samples failed to meet the USP limits for content assay, not because they did not have the active ingredients, but rather the API amounts fell short of the label claim. Majority of these samples were amoxicillin/ clavulanic acid oral dry suspensions.

Amoxicillin is generally more stable than clavulanic acid when dissolved in water (Mehta et al., 1994) thus seven days after reconstitution amoxicillin compared to clavulanic acid is expected to more stable. This was contrary for 6 of the amoxicillin/clavulanic acid oral dry suspension that failed content determination as depicted in Table 4.7, which were below specified limits for amoxicillin content on day seven yet within the limits for clavulanic acid. This reflects on the stability of the dry suspensions samples, which is an essential factor of quality, safety and efficacy of any drug product (Batchelor and Marriott, 2015). This could be attributed to factors such as poor storage conditions and / or poor manufacturing processes (Elena et al., 2014).

There was a significance difference in percentage chemical content for innovator products and the samples all across the data as shown in Tables 4.6, 4.7, 4.8 and 4.9. This could be attributed to the fact that these samples are from varied manufacturers who may be practicing different
manufacturing process and pharmaceutical products (Van der Schoot et al., 2007; Vulto and Jaquez, 2017).

4.6 Dissolution test

4.6.1 Dissolution data for amoxicillin capsules and amoxicillin/clavulanic acid tablet samples

All amoxicillin capsule (19) and tablet (4) samples and innovator product (control) investigated for dissolution of the API complied with the pharmacopoeia specifications, the data is given in Tables 5.0 and 5.1 respectively. According to the USP specifications percentage dissolution should be not less than 65% for amoxicillin and 70% for clavulanic acid (USP40-NF35, 2017a).
Table 5.1: Percentage dissolution test results for amoxicillin capsules sampled from various pharmacies in Nairobi County.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Country of Origin</th>
<th>Labelled active ingredients (mg)</th>
<th>Dissolution chemical content as % label claim (±RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAY52</td>
<td>China</td>
<td>250</td>
<td>99.00 (3.7)\text{(^a)}</td>
</tr>
<tr>
<td>SC10</td>
<td>Kenya</td>
<td>250</td>
<td>99.00 (1.3)\text{(^a)}</td>
</tr>
<tr>
<td>SB032</td>
<td>India</td>
<td>250</td>
<td>97.00 (2.5)\text{(^a)}</td>
</tr>
<tr>
<td>KAY56</td>
<td>India</td>
<td>500</td>
<td>97.80 (5.3)\text{(^a)}</td>
</tr>
<tr>
<td>KAY51</td>
<td>India</td>
<td>250</td>
<td>95.00 (1.8)\text{(^b)}</td>
</tr>
<tr>
<td>ZIM109</td>
<td>Kenya</td>
<td>250</td>
<td>94.00 (5.3)\text{(^b)}</td>
</tr>
<tr>
<td>CBD138</td>
<td>Kenya</td>
<td>250</td>
<td>93.00 (3.7)\text{(^b)}</td>
</tr>
<tr>
<td>KBR78</td>
<td>China</td>
<td>250</td>
<td>92.00 (1.7)\text{(^b)}</td>
</tr>
<tr>
<td>KAN120</td>
<td>United Kingdom</td>
<td>500</td>
<td>100.0 (2.5)\text{(^a)}</td>
</tr>
<tr>
<td>SC13</td>
<td>India</td>
<td>500</td>
<td>90.70 (2.3)\text{(^b)}\text{(^c)}</td>
</tr>
<tr>
<td>SB21</td>
<td>China</td>
<td>500</td>
<td>88.00 (1.8)\text{(^c)}</td>
</tr>
<tr>
<td>KBR84</td>
<td>Kenya</td>
<td>500</td>
<td>88.00 (3.8)\text{(^c)}</td>
</tr>
<tr>
<td>KBR87</td>
<td>India</td>
<td>250</td>
<td>87.00 (3.6)\text{(^c)}</td>
</tr>
<tr>
<td>KBR67</td>
<td>Kenya</td>
<td>250</td>
<td>84.00 (2.5)\text{(^d)}</td>
</tr>
<tr>
<td>KRN94</td>
<td>Mexico</td>
<td>500</td>
<td>82.00 (2.2)\text{(^d)}</td>
</tr>
<tr>
<td>KBR77</td>
<td>Kenya</td>
<td>500</td>
<td>82.50 (3.6)\text{(^d)}</td>
</tr>
<tr>
<td>CBD128</td>
<td>United Kingdom</td>
<td>500</td>
<td>82.00 (3.2)\text{(^d)}</td>
</tr>
<tr>
<td>KAY42</td>
<td>China</td>
<td>500</td>
<td>81.20 (2.5)\text{(^d)}</td>
</tr>
<tr>
<td>SC12</td>
<td>India</td>
<td>500</td>
<td>80.00 (3.9)\text{(^de)}</td>
</tr>
</tbody>
</table>

RSD-Relative Standard Deviation, USP limits: dissolution ~ not less than (NLT) 65% of label claim. Percentages (descending) with different superscript letters with respect to the innovator product on Table 4.9 are significantly different (p < 0.05).
Table 5.2: Percentage dissolution results for amoxicillin/clavulanic acid tablets sampled from various pharmacies in Nairobi County.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Country of Origin</th>
<th>Labelled active ingredients (mg)</th>
<th>Dissolution chemical content as % label claim (±RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innovator</td>
<td>United Kingdom</td>
<td>625</td>
<td>Clavulanic Acid: 100.00 (1.44)a, Amoxicillin: 94.00 (0.76)b</td>
</tr>
<tr>
<td>KAN095</td>
<td>India</td>
<td>375</td>
<td>Clavulanic Acid: 88.70 (3.40)c, Amoxicillin: 97.40 (2.80)a</td>
</tr>
<tr>
<td>CBD131</td>
<td>India</td>
<td>625</td>
<td>Clavulanic Acid: 77.60 (2.90)e, Amoxicillin: 88.10 (3.6)c</td>
</tr>
<tr>
<td>KBR090</td>
<td>India</td>
<td>625</td>
<td>Clavulanic Acid: 75.20 (1.61)e, Amoxicillin: 77.50 (1.97)e</td>
</tr>
<tr>
<td>SB057</td>
<td>India</td>
<td>625</td>
<td>Clavulanic Acid: 79.90 (2.90)e, Amoxicillin: 87.60 (2.20)c</td>
</tr>
</tbody>
</table>

RSD-Relative Standard Deviation, USP limits: Dissolution- NLT 70% for amoxicillin and NLT 65% for clavulanic acid of label claim. Percentages (descending) with different superscript letters with respect to the innovator product are significantly different (p < 0.05).

Drug dissolution is a prerequisite for drug absorption into the body and the rate is directly associated to the bioavailability (Shahrin, 2013; Shekunov and Montgomery, 2016). It is a tool that measures batch release ensuring batch to batch quality and constant quality during the drugs shelf life (Newton et al., 2006). Similar studies in Ghana, Nigeria and United Kingdom demonstrated compliance with pharmacopoeia requirements of all amoxicillin capsules tested (Kaur et al., 2015). However, this observation was somewhat inconsistent with different studies undertaken also in Nigeria and Ghana that reported 14.3% (1 out of 7) and 7.0% (1 out of 14) of amoxicillin/clavulanic acid tablets analyzed failed to complied with pharmacopoeia specifications (Olanrewaju et al., 2012; Nettey et al., 2014).
There was a significant difference (p < 0.05) when the dissolution for the innovator product on Table 5.1 and the capsules and tablets were compared. This is probably because these samples are products from varied manufacturers who may be practicing different GMP processes (Van der Schoot et al., 2007; Vulto and Jaquez, 2017).

4.7 Quality of samples in relation to country of origin and sample location

Samples from Nairobi Central (CBD) had the highest substandard prevalence at 9.4%, followed by Kibra (KBR) and South C (SC) at 5.7%, South B (SB) at 3.8%, Kayole (KAY), Kangemi (KGM) and Westlands (WST) at 1.9% each. Majority of the poor-quality drugs, 22.6% (12 out of 53) of samples as stated on the labels were manufactured in India, other countries include, China, Egypt, Jordan and France at 1.9% (1 out of 53) each.

Comparison of samples from the same manufacturers but with different batch numbers, gave varying results and is noted in Table 5.2. Some samples complied while others failed to meet the USP limits. Samples SC11, KRN93, SC03, CBD128, SC013 and KRN94 were from the same manufacturer but different countries of origin. This reflects poor adherence to good manufacturing and distribution practices hence compromising batch to batch quality (Orwa et al., 2008).
Table 5.3: Content determination and dissolution test data for samples from same manufacturers but differing in quality.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Origin</th>
<th>Sample type</th>
<th>Labelled active ingredients (mg)</th>
<th>Content Determination</th>
<th>Dissolution test</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAY56</td>
<td>India</td>
<td>Capsule</td>
<td>500</td>
<td>Complied</td>
<td>Complied</td>
</tr>
<tr>
<td>KAY43</td>
<td></td>
<td>Amoxicillin dry Oral suspension</td>
<td>125</td>
<td>Complied</td>
<td>N/A</td>
</tr>
<tr>
<td>KAN95</td>
<td></td>
<td>Amoxicillin/clavulanic acid tablets</td>
<td>375</td>
<td>Complied</td>
<td>Complied</td>
</tr>
<tr>
<td>CBD133</td>
<td></td>
<td>Amoxicillin/clavulanic acid oral dry suspension</td>
<td>228</td>
<td>Failed</td>
<td>N/A</td>
</tr>
<tr>
<td>KBR63</td>
<td>India</td>
<td>Amoxicillin dry Oral suspension</td>
<td>125</td>
<td>Complied</td>
<td>N/A</td>
</tr>
<tr>
<td>SC08</td>
<td></td>
<td>Amoxicillin/clavulanic acid oral dry suspension</td>
<td>125</td>
<td>Failed</td>
<td>N/A</td>
</tr>
<tr>
<td>SC11</td>
<td>France</td>
<td>Amoxicillin/clavulanic acid oral dry suspension</td>
<td>625</td>
<td>Failed</td>
<td>N/A</td>
</tr>
<tr>
<td>KRN93</td>
<td>Canada</td>
<td>Amoxicillin/clavulanic acid oral dry suspension</td>
<td>457</td>
<td>Complied</td>
<td>N/A</td>
</tr>
<tr>
<td>SC03</td>
<td>India</td>
<td>Amoxicillin dry Oral suspension</td>
<td>125</td>
<td>Failed</td>
<td>N/A</td>
</tr>
<tr>
<td>CBD128</td>
<td>New Zealand</td>
<td>Capsule</td>
<td>500</td>
<td>Complied</td>
<td>Complied</td>
</tr>
<tr>
<td>SC013</td>
<td>France</td>
<td>Capsule</td>
<td>500</td>
<td>Complied</td>
<td>Complied</td>
</tr>
<tr>
<td>KRN94</td>
<td>Mexico</td>
<td>Capsule</td>
<td>500</td>
<td>Complied</td>
<td>Complied</td>
</tr>
</tbody>
</table>

N/A - not applicable
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Amoxicillin is among the most easily available, most prescribed and affordable antibiotic in the Kenyan Market, yet little has been documented on its quality (WHO Expert Committee, 2017; Ewen and Okemo, 2018). A total of 106 different brands were identified in the market, majority of which were imports. Although all the samples had the active pharmaceutical ingredient, they did not comply with the other quality tests thus the drugs would likely be substandard. Dissolution studies for solid dosages; amoxicillin capsules and tablets showed 17.9% failure to comply with USP limits. Overall, 30.2% of samples analyzed were considered of poor quality according to USP requirements and were all imported products. This observation is contrary to the perception that imported compared to locally manufactured medicines in developing countries are of better quality. Almost all locations sampled registered presence of poor quality amoxicillin formulations. Therefore, ensuring quality of antibiotics will contribute to addressing costs of treatment due to anti-microbial resistance, reduction in disease burden through reducing treatment failure thus contributing towards universal health coverage which is a key target under the sustainable development goals and Kenya is among the governments that has pledged to achieve it by 2030.
5.2 Recommendations

i. There is need for regular post market surveillance to monitor the quality of drugs in the Kenyan market.

ii. There is need to adequately monitor good manufacturing and distribution practices of pharmaceutical manufacturers to ensure batch to batch quality.

5.3 Recommendations for further Research

There is need to extend the study to all the other counties in Kenya, especially Counties with entry points for drugs such as Eldoret, Kisumu and Mombasa.
REFERENCES


APPENDIX I: ETHICAL APPROVAL

KENYA MEDICAL RESEARCH INSTITUTE

TO: DR. BEATRICE IRUNGU
   PRINCIPAL INVESTIGATOR

THROUGH: THE DIRECTOR, CTMDR
   NAIROBI

Dear Madam,

RE: KEMRI/SERU/CTMDR/012/2059 (REQUEST FOR ANNUAL RENEWAL WITH A PROTOCOL DEVIATION REPORT): A SURVEY ON QUALITY OF COMMONLY PRESCRIBED ANTIBIOTICS IN KENYAN MARKET: A CASE STUDY OF AMOXICILLIN AND CO-TRIMOXAZOLE IN NAIROBI

Thank you for the continuing review report for the period April 9, 2018 to February 25, 2019.

This is to inform you that the Expedited Review Team of the SERU was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted approval.

This approval is valid from April 9, 2019 through to April 8, 2020. Please note that authorization to conduct this study will automatically expire on April 8, 2020. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the SERU by February 25, 2020.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SERU for review prior to initiation. You may continue with the study.

Yours faithfully,

ENOCK KEBENEI
THE ACTING HEAD
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health
APPENDIX 2: DATA SHEET

CODE __________LOCATION_______________________DATE____________________

Data Collector: Name______________________ Sign______________________

<table>
<thead>
<tr>
<th>Drug Name: AMOXICILLIN</th>
<th>Tablet</th>
<th>Capsule</th>
<th>Syrup</th>
<th>Suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand Names/ strength/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>country of origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Package Size (s)</th>
<th>Package Type</th>
<th>Package Size (s)</th>
<th>Type</th>
<th>Package Size (ml)</th>
<th>Type</th>
<th>Package Size (ml)</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>14</td>
<td>20</td>
<td>50</td>
<td>100</td>
<td>Other</td>
<td>Blister</td>
<td>Loose Pack</td>
<td>Other</td>
</tr>
<tr>
<td>Capsule</td>
<td>14</td>
<td>20</td>
<td>50</td>
<td>100</td>
<td>Other</td>
<td>Blister</td>
<td>Loose Pack</td>
<td>Other</td>
</tr>
<tr>
<td>Syrup</td>
<td>50</td>
<td>70</td>
<td>100</td>
<td>Other</td>
<td>Glass</td>
<td>Plastic</td>
<td>Glass</td>
<td>Plastic</td>
</tr>
<tr>
<td>Suspension</td>
<td>50</td>
<td>70</td>
<td>100</td>
<td>Other</td>
<td>Glass</td>
<td>Plastic</td>
<td>Glass</td>
<td>Plastic</td>
</tr>
</tbody>
</table>
APPENDIX 3: CONSENT FORM

A SURVEY ON QUALITY OF COMMONLY PRESCRIBED ANTIBIOTICS IN THE KENYAN MARKET: A CASE STUDY OF AMOXICILLIN IN NAIROBI

This study is being conducted by ……………………………….. and the Kenya Medical Research Institute. I kindly request you to read this form and ask any questions you may have before agreeing to participate in the study.

Introduction

Poor quality drugs are an important but often neglected public health problem particularly afflicting unsuspecting patients in ‘developing’ countries. It is a significant cause of unnecessary morbidity, mortality and loss of public confidence in medicines and health structures. Drugs may be of poor quality if they are counterfeit, substandard or degraded.

Purpose of study

You are therefore invited to participate in this study whose main objective is to determine the quality of some commonly prescribed antibiotics, amoxicillin products in Nairobi retail and hospital pharmacies. The information gathered from this study will be used to advise policy and to modify intervention programs which will go a long way in improving the medical status and the general quality of life of patients in Kenya at large.

Procedures to be followed

If you agree to take part in this study I will collect data from you. As a pharmacist, you will be asked to give

Benefits

The information gathered from this study will be used to modify intervention programs which can improve the health of the community.

Risks

There is no risk of participation in this study. You will not be expected to give your names to the person collecting data from you.

Assurance of confidentiality

The information you give which will be written down and will remain confidential and your name will not appear when we present this study or publish its results.

Storage of data
The data will be stored in secure cabinets and computers with password/s and will only be accessible to the investigators.

**Right to refuse or withdraw**

It is important that you understand the following general principles that will apply to all participants in the study:

1. Participation is entirely voluntary.
2. You may withdraw from this study at any time without penalty or loss of benefits.

Please feel free to ask any questions that you may have.

Do you agree to participate?

I acknowledge that this consent form has been fully explained to me in a language that I understand and had the opportunity to ask questions which have been answered to my satisfaction. I agree voluntarily to participate in this study and understand that I have the right to withdraw at any time without penalty.

Participant's name: ____________________________ *(Optional)*

Participant's signature: ______________________ Date: _______________

Study No: KEMRI/SERU

Investigator's signature: ______________________ Date: _______________

**Contact: Questions about research**

If you have any questions about this study, you may contact Dr. Beatrice Irungu at the Kenya Medical Research Institute, Nairobi Tel; 2722541 during the study and in the future.

If you have concerns about human rights, ethics and welfare issues you may contact the Secretary of the KEMRI Scientific and Ethics Review Unit;

Tel; 020-722541, mobile; 0717 719477

Email seru@kemri.org.