Screening the Efficacy of Commonly Retailed Antibiotics from Government and Private Pharmacies in Adama City, Oromia Regional State, Ethiopia

By: Demelash Demissie

Thesis Submitted to the Department of Applied Biology for the Award of Degree of Master of Science in Applied Biology (Microbiology)

School of Applied Natural Science

Office of Graduated Studies

Adama Science and Technology University

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June, 2018

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APPROVAL SHEET

This is to certify that the thesis prepared by Demelash Demissie Argachew entitled as "Screening the Efficacy of Commonly retailed Antibiotics from Government and Private Pharmacies in Adama City, Oromia Regional State, Ethiopia" submitted in partial fulfillment of the requirement for the master of degree of science in Applied Biology (Microbiology) complies with the regulation of the university and meets the accepted standards with respect to originality.

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DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged

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LIST OF ABBREVATIONS AND ACRONYMS

APF	Addis pharmaceutical factory plc
API	Active Pharmaceutical Ingredient
ASTU	Adama Science & Technology University
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
EPHARM	Ethiopian pharmaceutical manufacturing
EQA	External Quality Assessment
FMHACA	Food, Medicine and Health Care Administration and Control Authority
NCCLS	National Committee for Clinical Laboratory Standards
NQCL	National Quality Control Laboratories
OPHRCBQALC	Oromia Public Health Research Capacity Building and Quality Assurance Laboratory Center
PMS	Post-Marketing Surveillance
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Antibiotic resistance: resistance where a microorganism is able to survive exposure to an antibiotic

Batch number: refers to specific designation printed on the label of antibiotic that locates the batch and allows the production or manufacturing history of batch.

Bacteriostatic: work by stopping bacteria multiplying

Brand name: the trade name or the name chosen by the manufacturer.

Broad-spectrum: antibiotics affect a wide range of bacteria.

Counterfeit: deliberately and fraudulently mislabeled with respect to identity and/or source.

Efficacy: the power to produce a desired result or effect.

Generic name: the official antibiotic name for the active ingredient of the antibiotic.

Narrow-spectrum: antibiotics target specific types of bacteria.

Potency: refer to the quantity of antibiotic necessary to produce a given effect or it may refer to the maximum response which can be achieved with the antibiotic.

Shelf-life: refers to the period from initial preparation and packaging during which the drug dosage form continues to remain within its physical, chemical, therapeutic and toxicological specifications at specified storage conditions.

Substandard: refers to genuine products that have not passed the quality testing protocols previously set for each product

Abstract

Antibiotics lose their potency with poor storage and transportation earlier than the expiry date that appears on the label. In view of the sub-standards antibiotics produced by some pharmaceutical companies and counterfeit, antibiotic resistance is becoming a public health problem in recent times for both hospital and community acquired infections. The objective of this study was to screen the efficacy of commonly retailed antibiotics from government and private pharmacies in Adama city for their efficacy in treating diseases. The study employed prospective cross-sectional study techniques and data were collected and analyzed using EPINFO version 7 and SPSS 20 software. Interview for 42 participants (pharmacist and druggist) to obtain antibiotic information using structured questionnaires. A disc diffusion antibiotic susceptibility test was used to screen a total of 162 antibiotics produced by different manufacturers (both local and foreign) purchased from government and private pharmacies against standard strain of bacteria based on the Clinical and Laboratory Standards Institute (CLSI) guideline in OPHRCBQALC. This study revealed that of the total evaluated antibiotics 52.4% of amoxicillin, 66.7% of ciprofloxacillin, 31.6% of azithromycine and 100% of ceftriaxone passed efficacious test. The result of this study showed, significant association between local source and inefficacious status of amoxicillin (x^2=4.7, p< 0.03). There was also statistically significant association between pharmacy ownership and efficacious of ciprofloxacillin (x^2= 7.4, p< 0. 007). Therefore, government should initiate surveillance of antibiotics efficacious test to strengthen the production of good quality antibiotics, regulatory bodies should monitor antibiotics quality at dispensing area and at production site regularly and this study recommends further works on quantitative based antibiotics studies.

Keywords: antibiotics, efficacious, resistant, susceptibility test

1. INTRODUCTION

1.1 Background

Antibiotics are type of antimicrobial agents used in the treatment and prevention of bacterial infections. Since the introduction of the first antibiotics, they become one of the cornerstones of modern medicine and without antibiotics, many of the now widely used therapies and medical procedures would not be possible (White, 2011).

Antibiotics require controlled storage and transit conditions in order to ensure that their quality is not compromised (Wondimeneh *et al*., 2014). Proper environmental control (i.e. proper temperature, light, humidity, conditions of sanitation, ventilation, and segregation) must be maintained wherever antibiotics are stored in the premises (Kausar *et al*., 2013). Counterfeit antibiotics are deliberately and fraudulently mislabeled with respect to identity, composition and source because of weak regulatory systems (Ratanawijitrasin and Wondemagegnehu, 2002). This term can apply to both generic and branded antibiotics and may include correct ingredients, wrong ingredients, no active pharmaceutical ingredients (APIs), insufficient ingredients, and which are typically sold with fake labeling and fake packaging (Newton *et al*., 2006).

The problem of counterfeiting is difficult to detect, investigate, and quantify. It simply means that it is hard to know or even estimate the true extent of the problem. It is estimated that counterfeit antibiotics account for nearly 28% of global counterfeit pharmaceutical products which include the "old" antibiotics such as beta lactams which have commonly been used for years (Kelesidis *et al*., 2007). Substandard antibiotics are genuine products produced by manufacturers authorized by the national medicine regulatory authority which do not meet quality specifications set for them by national standards (WHO, 2014). A mild difference in the concentration of active ingredient in antibiotic preparation may have impact in actual efficacy including allergic reactions, unexpected side effects, poisoning, untreated disease and early death (Delepierre et al., 2012).

On the other hand, using inaccurate concentration of antibiotics lead to the resistance of sensitive bacteria, in addition to weakening the immunity system in some human due to poor nutrition or heredity factors make bacteria to be more resistant (Mahmood and Ahmed, 2008). Antibiotic resistance is the ability of bacteria to withstand the effects of an antibiotic. Antibiotic resistance occurs when bacteria change in a way that reduces the effectiveness of antibiotics designed to

cure or prevent infections. The bacteria survive and continue to multiply, causing more harm. There is certain mechanism of antibiotic resistance of bacteria, one of its emerging is antibiotic resistance genes on chromosome or on plasmids. It is well known that plasmids are major vectors for the dissemination of both antibiotic resistance and virulence determinants among bacterial populations. The exchanging of genetic materials between microorganisms through transformation, conjugation or transduction processes or may be by mobile genes (transposons) have been proposed as a major contributor in the rapid evolution of microorganism's resistance to antibiotics (Mahmood and Ahmed, 2008).

The prolonged treatment of diseases and infections due to antibiotic resistance causes increased health costs and serious implications for human welfare, a serious public health problem, particularly in emerging economies and developing countries, and may have a significant impact on the national, clinical and economic burden (Johnston and Holt, 2014).

In this study, the antibiotic efficacy of amoxicillin, ciprofloxacillin, azithromycin and ceftriaxone antibiotic samples purchased from pharmacies in Adama city were evaluated against reference strains of bacteria using the Kirby-Bauer disk diffusion method. Acceptance of the *in vitro* disc susceptibility test method has been aided by its simplicity and rapidity which is recommended by CLSI (CLSI, 2016). This method of antibiotic susceptibility test has been described as a reliable, easy and inexpensive method of evaluating antibiotic efficacy (Manoharan *et al*., 2003).

1.2 Statement of the problem

Antibiotics play an important role in maintaining health, preventing disease and saving lives. However ineffective antibiotics pose great risks to individuals and even threaten lives in emergencies (Keoluangkhot *et al*., 2008). Ineffectiveness takes several forms, such as antibiotics containing less than the stated dose of the active ingredient or containing unstated or harmful substance(s). Similarly, antibiotics that have been degraded or adulterated due to improper storage and handling may be ineffective (Gaudiano *et al*., 2007). Degradation occurs in antibiotics much before they approach the expiry dates put on the packs due to uncontrolled temperature, exposure to light and moisture, and when they are transported from the manufacturing site to retail pharmacies (Unnikrishnan, 2008). The loss of potency during storage may influence the efficacy and safety of antibiotics (Kausar *et al*., 2013). Antibiotics should be stored in a specially designed secure area or space of a building in order to avoid contamination or deterioration, disfiguration

of labels, maintains integrity of packaging and so guarantee quality and potency of antibiotics during shelf life (Akrayi and Abdulrahman, 2013).

Poor-quality antibiotics can also reach the market through substandard production of legitimate antibiotics due to inadequate quality-control processes during manufacture, as well as by deliberately fraudulent practices (Johnston and Holt, 2014). The wide use of antibiotics as a result of poor quality in the treatment of bacterial infections has led to the emergence and spread of resistant strains. The emergence of multiple drug resistant bacteria has become a major cause of failure of the treatment of infectious disease (Olowe, 2012) and resistant infections are more difficult to treat, can lead to long-term illness, increased healthcare costs, the need for surgery to remove infected tissue and even death (Unnikrishnan, 2008). Poor quality of antibiotics can also facilitate allergic conditions and cause decay which results in stomach or kidney inflammations due to the fermented protein content of an improperly stored antibiotic (Hedwig, 2007).

Expansion of pharmaceutical industries in many countries with advancement in transport technologies facilitated not only trade of genuine pharmaceutical products but also the circulation of poor quality and counterfeited antibiotics in developing countries (Kelesidis et al., 2007). Adama city is situated along the road that connects Addis Ababa with Dire Dawa. A large number of trucks use this same route to travel to and from the seaports of Djibouti and additionally, the new Addis Ababa-Djibouti Railway runs through Adama and this makes the city one of the busy city in the country with strong business activities.

Various reports including state media, Oromia Regional Custom and Revenue Authority Office indicated that many kinds of illegal commodities including antibiotics might be sold in private and government pharmacies found in Adama city and distributed to consumers and this might be impacting the health of the community negatively and as a consequence of such damaging effects, the community may lose confidence on health facilities and it may erode the suppliers and sellers of genuine antibiotics, the pharmaceutical industry, and it may drain household budget and national treasuries.

1.3 Objectives

1.3.1 General objective

The general objective of this study was to screen some commonly retailed antibiotics in governmental and private pharmacies found in Adama city for their efficacy in treating diseases.

1.3.2 Specific objectives

- To determine the proportion of poor quality antibiotics sold in private and government pharmacies in Adama city.
- To determine factors for antibiotic inefficacies

1.4 Hypothesis

The commonly sold antibiotics in private and governmental pharmacies found in Adama city are efficacious.

1.5. Significance of the study

The study has been planned to screen the efficacy of commonly sold antibiotics from private and governmental pharmacies found in Adama city and study in this area help to understand the magnitude of the problem, by understanding the problem the risk for increased morbidity and mortality associated with antibiotic ineffectiveness can be reduced, significant cost savings can occur , and this has a tremendous benefit for healthcare system and it also increase community confidence in healthcare systems and healthcare professionals. The study is also useful to fill the existing gap (absence of conducted research on efficacious of antibiotics) and can provide information for the Quality Control Units of the Ethiopia Food, Medicine, Health Administration and Control Authority which are charged with this responsibility. It can also serve as a reference for other researcher for further study on efficacy of antibiotics and being the first in the history of Adama city the study is thought to be important in different dimension.

1.6. Limitation of the study

The study has not been assisted by other methods like quantitative study for better conclusion due to inadequate resources and funds. Another limitation was pharmacy owners were not willing for the interview and giving samples for the study by assuming that the finding of the study may impose to close their pharmacy by the regulatory body.

2. LITERATURE REVIEW

In a recent literature review, 44%, 30%, and 9% of 163 counterfeit antibiotics were detected in Asia, Africa, and Europe and North America, respectively. Overall, up to 60% of antibiotics in Africa and Asia may have low quality (Delepierre *et al.*, 2012). Canada's regulatory agency reported that over the nine-year period to 2013, close to 650 products were deemed to be substandard and 10 % of them were antibiotics (Almuzaini *et al*., 2013). Another study conducted in UK, out of 269 cases, 13 % of them were substandard antibiotics (Almuzaini *et al*., 2013).

A study conducted in Southeast Asia revealed that 38% of antibiotics on sale in pharmacies did not contain any active ingredient and resulted in a number of preventable deaths (WHO, 2014). Another study conducted in 16 countries of Africa and Asia on amoxicillin and benzyl penicillin shows that the percentage of failed samples from unlicensed outlets was 51% but only 24% in licensed outlets. Of the 15 studies identified, 93% found inadequate amounts of active pharmaceutical ingredients (APIs) 47% found an absence of active pharmaceutical ingredients, and 33% found dissolution failure (Almuzaini *et al*., 2013). A survey of amoxicillin in the capital of Papua New Guinea found all samples outside of pharmacopeial specifications; 14 % had undetectable levels of active ingredient (Nair *et al*., 2011).

A survey in Egypt, Jordan, Lebanon, and Saudi Arabia found more than half of antibiotics sampled to be substandard (Kyriacos *et al.*, 2008). Another study explored the Active Pharmaceutical Ingredient (API) content of the antibiotic amoxicillin purchased from Egypt, Lebanon, Jordan, and Saudi Arabia show that more than 50% of samples had lower Active Pharmaceutical Ingredient than accepted by pharmacopeial limits, and therefore were considered substandard (Kyriacos *et al.*, 2008).

A study show that antibiotics in India lose 3-4% of their potency at pharmacy because they are not stored properly (Unnikrishnan, 2008). Nearly 8,000 deaths over five years in the Indian state of Jammu and Kashmir were linked to an antibiotic that contained 0 mg of amoxicillin, instead of the stated 500 mg (Wani, 2013). Another study conducted on amoxicillin show that sample which was obtained from Iraq was found to be counterfeit and did not contain any amoxicillin at all (Idrees, 2009). Several studies have reported the presence of substandard and counterfeit antibiotics in Cambodian pharmaceutical markets, with prevalence ranging from 4% to 90%

(Khan *et al.,* 2013) and study conducted to evaluate the quality of amoxicillin–clavulanic acid preparations under tropical conditions show that 16.9% samples failed quantity and content uniformity and 33.9% failed dissolution tests (khan *et al.*, 2013)

A random sample of all known pharmacies in three districts of Ghana found the antibiotics to be of uniformly poor quality 39 % of the samples tested were below British Pharmacopoeia specifications though only 2 % were expired (Stanton *et al.*, 2012). A study of generic versions of rifampicin tablets found that, on initial inspection, four of 17 samples (24%) did not meet the label specifications for rifampicin content. After 3 months of storage in temperature-stressed conditions (40°C and 75% relative humidity), a further four samples (total 47%) failed to meet the content specifications (Angeli and Trezza, 2009). Studies conducted in Burma, Cameroon, Vietnam, Cote d'Ivoire, and Nigeria, between 8% and 35% of the antibiotics were found to be counterfeit, in most cases with the active pharmaceutical ingredient content being out of the pharmacopoeial limits and with no active pharmaceutical ingredient present (Okeke *et al.*, 2009). A Rwandan study on antibiotics stability found that 20 % of antibiotics in a sample of Kigali and Butare pharmacies were substandard at the time of purchase (Twagirumukiza *et al.*, 2009). A random survey by the National Quality Control Laboratories (NQCL) and the Pharmacy and Poisons Board found the almost 30% of drugs in Kenya are counterfeit (Angeli and Trezza, 2009).

In Ethiopia, the 2008 report of WHO on the estimation of the proportion of counterfeiting by category revealed that 12% was antibiotics and Amoxicillin was one of the most susceptible antibiotics for counterfeiting because of its high demand in the market (Lantider, 2015). The result of trend analysis on the quality control laboratory tests carried out from the year 2007–2011 shows that most failures of samples submitted for post-marketing surveillance (PMS) was higher 9.5%–15.5% than samples submitted for the purpose of marketing authorization 4.7% – 10.7% (EFMHACA, 2012). Another national quality survey study conducted in Ethiopia reported that a significant proportion of poor quality tinidazole tablets which account 29% was substandard (Suleman *et al.*, 2014).

3. MATERIALS AND METHODS

3.1. Description of study area

This study was conducted from March 2018 to June 2018 on commonly sold antibiotics from Government and Private pharmacies found in Adama city, Oromia regional state, Ethiopia. The city forms a Special Zone of Oromia and is surrounded by East Shewa Zone. It is located 99 km Southeast of Addis Ababa at 8.54°N 39.27°E with an altitude of 1712 meters above sea level between the base of an escarpment to the west, and the Great Rift Valley to the East. Its annual average temperature and rainfall are 20.5^{o}C and 809mm respectively (Anon, 2011). The city covers an area of 13,666.5 hectares (133.6-km^2). The population is more than 356,344 of which 176,487 are male and 179,857 are female (CSA, 2015) with heterogeneous ethnicity. Trade along with investment is one of the key aspects of the city's economic life. In this city there are 42 pharmacies of which eight governmental, thirty-four private health facilities and also regional Public Health Research, Capacity Building and Quality Assurance Laboratory center where different referred sample are tested with in the region.

3.2. Materials

The following materials and reagents were used during screening the effectiveness of commonly retailed antibiotics in governmental and private pharmacies found in Adama city.

- Incubator
- Biosafety cabinet
- Refrigerator
- Petri dish
- Sterile Inoculating swab
- Sterile inoculating loop
- Autoclave
- Antibiotics disc
- Muller Hinton Agar (MHA)
- Distil water
- Stove with magnetic stirrer
- Whatman Filter paper
- Puncher
- Micropipettes
- ATCC organism
- Personal protective equipment
- 95% Ethanol
- Phosphate buffer
- Broth media (nutrient broth)

3.3 Study design

A prospective cross-sectional study technique was employed using selected structured interview and observation for screening the effectiveness of commonly retailed antibiotics in government and private pharmacies found in Adama city.

3.3.1 study population

A study population comprised of all registered governmental and private pharmacies found in Adama city were eligible for inclusion and exclusion of antibiotics retailed in the drug store and clinics.

3.4. Study variables

Both dependent and independent variables were used. The dependent variable was antibiotic efficacy. The independent variables include categorical variables including type of pharmacy (community pharmacy, hospital pharmacy and health center pharmacy), educational level of dispensers (druggist, pharmacy technician and chief pharmacist), antibiotic storage conditions (temperature monitoring, ventilation conditions, and shelf life). Additionally, other variables like antibiotic transport conditions, expiry date, manufacturer origin and packaging conditions were considered.

3.5. Measurement and Data collection

3.5.1. Sample size

The required sample size for this study was determined based on the accessibility and avaibility of pharmacy in the Adama city. From 42 available pharmacies (34 private and 8 governmental) a duplicate of amoxicillin, ciprofloxacillin, azithromycin and ceftriaxone were randomly selected and tested for their efficacies.

3.5.2. Sampling Techniques

All 42 Pharmacies (private and governmental) were included in the study but the antibiotic was selected using simple random sampling techniques from each study group of antibiotic.

3.5.3. Data collection procedure

3.5.3.1 Structured interview

Structured interviews were administered to capture the sociodemographic characteristics of druggists and pharmacists working in the target pharmacies. From each pharmacy one study participant (druggist or pharmacist) was interviewed twenty-three structured questions to capture sociodemographic characteristics and information about the antibiotic at the practice setting of the respondents (AnnexII).

3.5.3.2 Structured Observation

The observation period was confined during data collection time and the availability of some measuring instruments and facilities like thermometer, ventilator, refrigerator, light protector, batch number, manufacturer's origin and date of expiry were examined and recorded on the structured observation checklist as indicated in Annex II.

3.5.3.3. Laboratory test

For the purpose of this study, four different antibiotic groups (amoxicillin 42 strips, ciprofloxin 42 strips, azithromycin 38 strips and ceftriaxone 42 vials) with a total of 164 antibiotics (single strip contain 10 tablets in amoxicillin and ciprofloxacin but it contains three tablets in case of azithromycine) were purchased from thirty-four private and eight governmental pharmacies found in Adama city. All the samples were checked for their batch numbers, manufacturer's origin and date of expiry and delivered to Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory center (OPHRCBQALC). The disc diffusion of antibiotic before used as a method, it was verified by replicating 3 time each disc for 5 consecutive test days (3x5days) in the laboratory (CLSI, 2016) its precision (standard deviation) for amoxicillin, ciprofloxacillin, azithromycine and ceftraxione were 2.364,3.364,1.63 and 1.67 and the uncertainty measurement with a 95% CI were, (17-19), (31-32), (22-24) and (31-33) respectively. Regard to the CLSI disc diffusion test break point, all antibiotics inhibition zone range were acceptable (CLSI, 2016). While performing the antibiotic sensitivity test, 500mg of ciprofloxacin tablet dissolved in 500ml of distilled water, and 1000mg of injectable powder ceftriaxone in 166.7ml of distilled water whereas 500mg of azithromycin tablet first dissolved in a 5ml of 95% ethanol with a broth media and the amoxicillin also first dissolved and diluted in a phosphate buffer of pH- 6 (Table 1) and by taking 5µl from each dissolved stock antibiotics(i.e. amoxicillin (10 µg), ciprofloxacin (5 µg),

azithromycin (15 µg), ceftriaxone (30 µg) impregnated on the discs of 6.0 mm diameter and a disc with sterilized distil water was set as the negative control.

Table 1. Solvent and diluent for preparation of stock solution of antibiotic agent

S.no	Antibiotics capsule, tablet and powder	Solvent	Diluent
		Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antibiotics capsule, tablet and powder	Finishing diluting the final stock solution as stated below
1	Amoxicillin 500mg	Phosphate buffer PH-6.0, 0.01mol/L	Phosphate buffer PH-6.0, 0.01mol/L
2	Ciprofloxacillin 500mg	water	water
3	Azithromycine 500mg	95% of ethanol or glacial acetic acid(1/2 water, glacial acetic acid not greater than 2.5µl/ml	Broth media
4	Ceftraxione1000mg	Water	water

Source: CLSI guideline M_100S26, 2016 G.C, 26 ed, Table 6A, page 192.

Table 2. Antibiotics dilution, concentration and dispensing volume preparation

Antibiotics	G	D	C=G/D	P	S=p*c
Amoxicillin	500mg	250ml phosphate buffer PH-6	2mg/ml=2µg/ µL	5 µL	$10\mu g$
Ciprofloxacillin	500mg	500ml	1mg/ml=1µg/ µL	5 µL	$5\mu g$
Azithromycin	500mg	161.7ml+5ml of 95% ethanol=166.7ml	2.9mg/ml=2.9µg/ µL	5 µL	$15\mu g$
Ceftraxione	1000mg	166.7ml	5.9mg/ml=5.9µg/ µL	5 µL	$30\mu g$

$$D = \frac{P * G}{S}$$

$$C = \frac{G}{D}$$

Where

- D=Diluent volume to prepare standard concentration
- P=dispensing volume on the disc
- G=gram of antibiotic purchased from pharmacies
- S=standard disc concentration based on CLSI 2016
- C= prepared concentration of antibiotics

Mueller-Hinton Agar was prepared by cooling the medium after autoclaving at the temperature of 121ºC for 20 minutes. Discs of diameter 6.0 mm was punched from a sheet of Whatman Number 3 filter paper (UK) by a perforator and arranged in Petri dishe allowing a distance of 24 mm between each of them. The discs were sterilized in an oven at 121°C for 15 min. As a control 4 discs were randomly selected and placed on Mueller Hinton Agar in a petri dish and incubated at 37.0°C for overnight. The discs were allowed to cool (to room temperature) and the impregnated discs were arranged in separate petri dishe and dried by placing in an incubator at 37°C for 16-24 hrs. American Type Culture collection standard reference strains *Escherichia coli* (*E. coli*) ATCC-25922 and *Staphylococcus aureus* (*S. aureus*) ATCC-25923 were used for antibiotics susceptibility testing. The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method as recommended by CLSI using Mueller-Hinton agar (CLSI, 2016). For good standardization to attain reproducible results, the following measures were taken during the susceptibility testing: the disc diameter was uniform,6.0mm in diameter, the volume of agar poured to each plate and the sizes of the dishes were uniform (15mm by 150cm), bacterial cells use as inoculum were adjusted to 0.5 MacFarland. The antibiotic susceptibility profiles were interpreted based on Clinical and Laboratory Standards Institute guidelines (CLSI, 2016). After inoculation for about 15 min, each of the impregnated discs were placed gently onto the inoculated agar. The distance between each impregnated disc on agar plate was made about 5cm and approximately 15 mm from edge of plate as described by (Clutterbuck *et al.*, 2007). The agar plates were incubated for 16-18hrs at 37°C. Zone of inhibition was measured using ruler in mm to

determine the microorganism's sensitivity to the antibiotics. All the tests experiments were conducted in triplicates and the means were recorded to determine efficacy of antibiotics. If the measured values were between the standard, the antibiotic is categorized as potent. The results were recorded and the images were captured.

3.6. Data Quality Assurance

For antibiotics efficacy test, culture media, reagent, and antibiotic disc were used and the entire samples that are processed in the laboratory were in accordance with the updated microbiology guideline. The laboratory also has ATCC organism and External Quality Assessment (EQA) isolate organism. All these ensure that the laboratory is providing quality service. Additionally, pretest of the study is checked to ensure the applicability of the study and compatibility of the variable during data collection to avoid measurement error.

3.7. Data analysis

Data were analyzed using EPINFO 7 and subsequently analyzed using a Statistical Package for Social Sciences (SPSS) version 20. Frequencies and percentage were used to describe the study population in relation to the relevant variables. Odds ratios, 95% confidence intervals, and p-values were computed to assess the presence and degree of association between antibiotic efficacy, antibiotics handling conditions and demographic characteristics. The Chi-square test was used to compare proportions (pharmacy ownership, work experience, awareness on illegal antibiotic traders, air conditioning and shelf life) with the efficacious of antibiotics and a p-value of <0.05 was considered statistically significant.

3.8. Ethical considerations

Ethical clearance was obtained from Health Research Ethical review committee of Oromia Health Bureau (Annex III). Permission for data collection was obtained from Adama city administration health office (Annex IV) and Oromia Public Health Research Capacity Building and Quality Assurance Laboratory was requested for experimental laboratory activities. The purpose and procedures of the study was explained to the owner of the pharmacy or pharmacists, with in the study period. All the information obtained from the study participants were kept confidential.

3.9. Dissemination of Result

The study result was presented to the Department of Applied Biology, School of Applied Natural Science, ASTU as MSc thesis and the result was also submitted to Oromia Health Bureau and FMHACA.

4. RESULTS

4.1. Profile of antibiotics retailed in Adama city

The most commonly prescribed antibiotics by the physicians in Adama city were Amoxicillin, ciprofloxacillin, azithromycine and ceftriaxone which make up 76.2% as shown in fig.1.

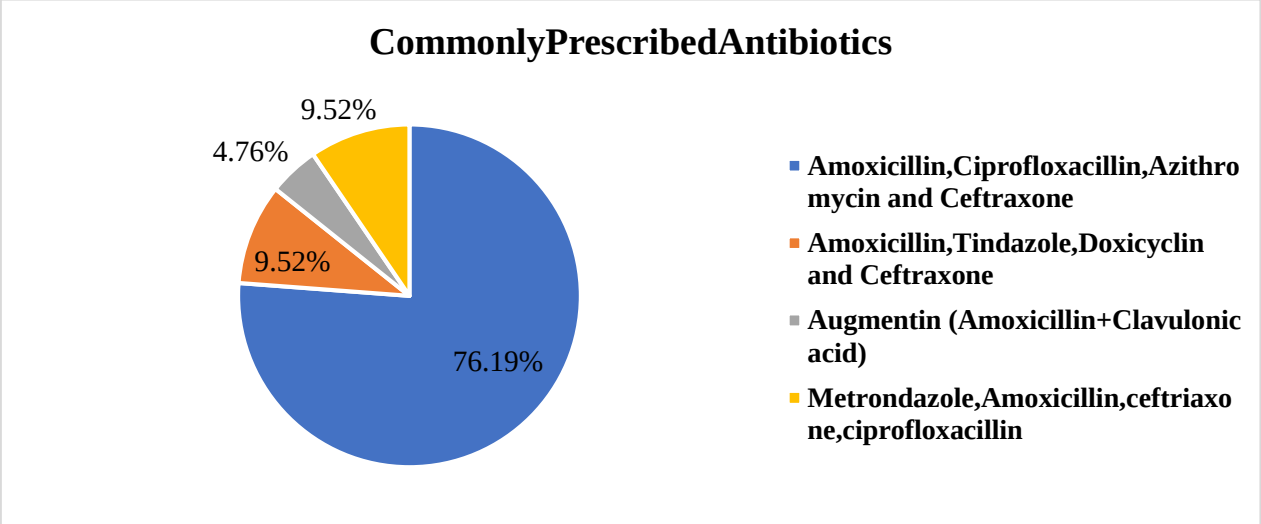

Figure 1. Comparison of most commonly prescribed antibiotics in Adama city, 2018.

Four different antibiotic groups with a total of 164 single antibiotics were used for this study (Table 3). Among this 9.12 % and 9.76 % of amoxicillin produced by APF and EPHARM manufacturer respectively. Ciprofloxacin 6.1% of them were produced by Huonsco Ltd and Sandoz a Novartis Company manufacturer. Regarding azithromycine antibiotics 5.49 % and 4.88 % were produced by Cadila Pharmaceuticals and Zim laboratories ltd manufacturer respectively. Ceftriaxones antibiotics 7.93 % and 5.49 % were produced by Shandonglukang and CSPC ZhougnuoPharm manufacturers respectively.

Table 3. Frequency of antibiotics based on manufacturers from Adama city (n=164),2018.

Antibiotics	Manufacturer	Frequency	percent
Amoxicillin	APF	15	9.12
	Denk-Pharma	1	0.61
	EPHARM	16	9.76
	GlaxoSmithKline	3	1.83
	Ipca Labor	3	1.83
	Kopra ltd	3	7.1
	Remedica	1	0.61
Ciprofloxacin	APF	4	2.44
	Brawn Lab	1	0.61
	Cadila Pharmaceutical	4	2.44
	EPHARM	6	3.66
	Huonsco Ltd	10	6.1
	Leben laboratories.	7	4.27
	Sandoz a Novartis Company	10	6.1
Azithromycin	Bafna Pharmaceutical Ltd	1	0.61
	Beximco pharmaceutical Ltd	7	4.27
	Cadila Pharmaceuticals	9	5.49
	Coral Laboratories Ltd	2	1.22
	Corporation Ltd	1	0.61
	Deva Holding	5	3.05
	EPHARM	1	0.61
	Sandoz aNavartis company	2	1.22
	sherya life science	1	0.61
	Umedica Laboratories Ltd	1	0.61
	Zim laboratories ltd	8	4.88
Ceftriaxone	Ashish Life Science	6	3.66
	AsralSteriTechPv.Ld	5	3.05
	BilimPharmaceutical	2	1.22
	CSPC ZhougnuoPharm	9	5.49
	Gulf Pharmaceuticalind	3	1.83
	Shandonglukang pharmaceutical	13	7.93
	Theon Pharmaceutical	2	1.22
	VHB medi science ltd	1	0.61
	Zhuhai Kinhoo pharmaceutical	1	0.61
Total		164	100.0

EPHARM=Ethiopian pharmaceutical manufacturing, APF=Addis pharmaceutical factory plc

4.2. Source of antibiotics

The antibiotics investigated in this study were manufactured by various countries. Majority of (73.8%) amoxicillin, 33.3% of ciprofloxacillin and 26.3% of azithromycine were purchased from local source whereas 14.3% of amoxicillin, 19% of ciprofloxacillin, 34.2% of azithromycine and 33.3% of ceftraxione were purchased imported from India. Nearly half of (54.8%) ceftraxione were purchased imported from China. Substantial proportion of the antibiotics were purchased imported from Romania, South Korea, Bangladesh and Turkey (Fig. 2).

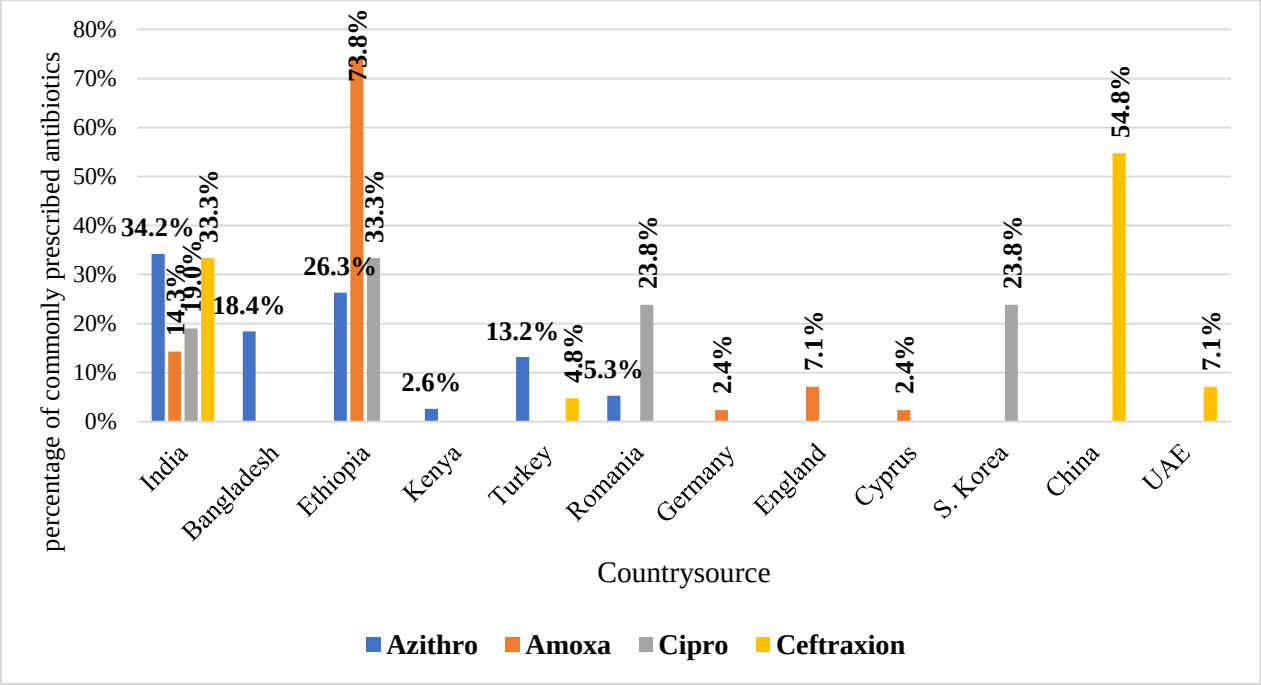

Figure 2. Comparison of most commonly prescribed antibiotics based on sources sold in Adama city, 2018.

4.3. Evaluating potency of antibiotic retailed in Adama city

In order to evaluate the potency of antibiotics *E. coli* (ATCC-25922) and *Staphylococcus aureus* (ATCC-25923) were used as standard microorganism. The sensitivity test and zones of inhibition around every disc were calculated in millimeter for amoxicillin, ciprofloxacin, azithromycin and ceftraxione antibiotics purchased from the private and governmental pharmacies found in Adama city.

4.3.1. Amoxicillin efficacious test

As indicated in Table 4, the three amoxicillin manufactured by kopra Limted-India, met the standard inhibition zone (15-22mm) with average inhibition zone of 21.33 ± 2.3. Amoxicillin manufactured by Denk Pharma-Germany and Remedica-Cyprus have mean inhibition zone of 15±0. Whereas amoxicillin manufactured by APF-Ethiopia, GlaxoSmithKline-England and Ipca Labor-India manufacturer have mean inhibition zone 14.7± 3.2. The minimum mean inhibition zone obtained in this study was 11.88 ± 4.7 manufactured by EPHARM-Ethiopia. The maximum mean inhibition zone obtained was 21.33 ±2.3 manufactured by Kopra limted-India.

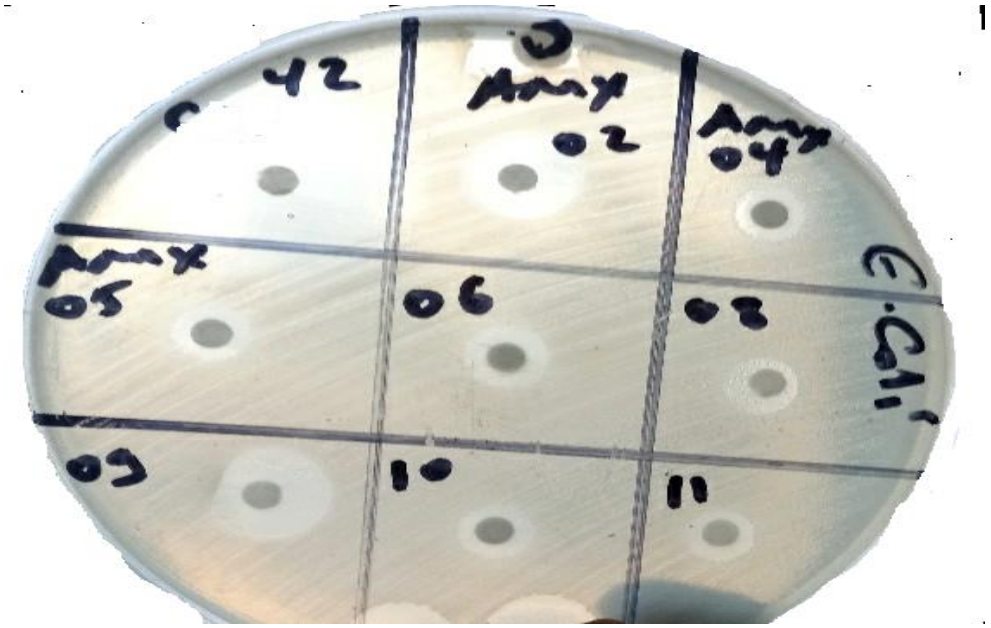

Figure 3. Zone of growth inhibition observed by amoxicillin using strain *E. coli* ATCC25922.

The above figure indicates that the amoxicillin inhibition zone measurement and the code label on each discs substitute the name of the pharmacy where the antibiotics were collected

Table 4. Mean inhibition zones of amoxicillin obtained from various manufacturers on *E. coli* ATCC-25922 organism,2018.

ATTC organism	Amoxicillin Manufacture	n	Mean ZI (mm±SD)	±95% CI	Standard range(mm)
E.coli	APF-Ethiopia	15	14.7±3.4	1.7	15-22
	Denk Pharma-Germany	1	15.0 ±0.0	0.0	
	EPHARM-Ethiopia	16	11.9±4.7	2.3	
	GlaxoSmithKline-England	3	14.7± 3.2	3.6	
	Ipca labor.-India	3	14.7±3.2	3.6	
	Kopra Limted-India	3	21.3±2.3	2.6	
	Remedica-Cyprus	1	15.0±0.0	0.0	

SD: Standard deviation**, ZI**: Zone of inhibition**, CI**: confidence interval, NB: each value was the mean of three readings **n**; number of samples.

As shown in Table 5, amoxicillin produced by different manufacturers with different brand name showed different efficacious results. Among 42 amoxicillin used for efficacious test 52.4% were passed efficacious test and 47.6% were failed. From those manufacture listed below, all amoxicillin manufactured by Kopra limted-India with AMYN 500 brand name, Remedica-Cyprus with Amoxapen 500 brand name and Denk Pharma with Amoxi-Denk brand name were passed the efficacious test. The highest amoxicillin failure observed in Ipca Laboratory-India (66.7%), APF-Ethiopia (60%), and EPHARM-Ethiopia (50%) with brand name of Imox, Amoxid 500 and Amoxicillin 500, respectively.

Table 5. Efficacious status of amoxicillin antibiotics based on their manufacturers and brand name in Adama city,2018.

Efficacious status of Amoxicillin with manufacturer and brand name								
Manufacturer	**country**	**Brand name**	**Conc**	**Pass**		**Fail**		**Grand Total**
				n	**Percent**	**n**	**Percent**	
APF	Ethiopia	Amoxid	500mg	6	40	9	60	15
Denk Pharma	Germany	Amoxi-Denk	500mg	1	100	0	0	1
EPHARM	Ethiopia	Amoxicillin	500mg	8	50	8	50	16
GlaxoSmithKline	England	Amoxil	500mg	2	66.7	1	33.3	3
Ipca laboratory	India	Imox	500mg	1	33.3	2	66.7	3
Kopra limted	India	AMYN	500mg	3	100	0	0	3
Remedica	Cyprus	Amoxapen	500mg	1	100	0	0	1
Grand Total				**22**	**52.4**	**20**	**47.6**	**42**

EPHARM=Ethiopian pharmaceutical manufacturing, APF=Addis pharmaceutical factory plc, mg=milligram, Conc=Concentration, n=Number

4.3.2. Ciprofloxacillin efficacious test

As can be seen from Table 6, ciprofloxacillin manufactured by APF-Ethiopia with mean inhibition zone of 34±7.1,Cadila Pharmaceutical- Ethiopia with mean inhibition zone of 34 ± 2.8, EPHARM-Ethiopia with average inhibition zone of 32.5 ± 4.6, Brawn Lab-India with mean inhibition zone of 32mm and Huonsco Ltd- South Korea with mean inhibition zone of 31.1 ± 1.9 were met the standard inhibition zone (30-40mm) but ciprofloxacillin manufactured by Leben Lab-India and SANDOZ aNavartis-Romania have mean inhibition zone of 26.3± 8.2and 29.1± 3.5 respectively. The minimum and maximum mean inhibition zone were 26.3 ± 8.2 and 34±7.1, respectively.

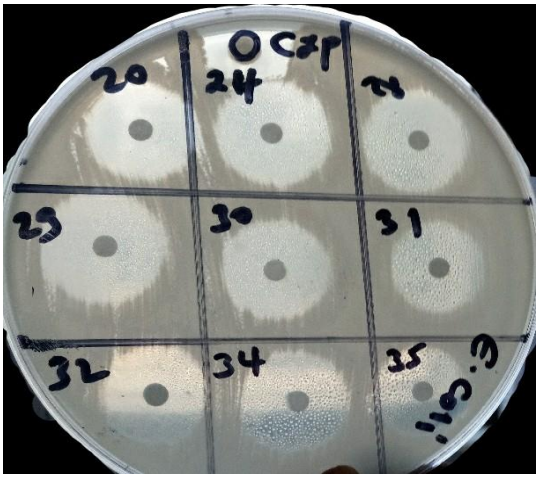

Figure 4. Zone of growth inhibition observed by ciprofloxacin using strain *E. coli* ATCC25922.

The above figure indicates that the ciprofloxacillin inhibition zone measurement and the code label on each discs substitute the name of the pharmacy where the antibiotics were collected

Table 6. Mean inhibition zones of ciprofloxacillin obtained from various manufacturers on E. coli ATCC-25922 organism in Adama city,2018.

ATTC organism	**Ciprofloxacillin Manufacturer**	**n**	**Mean ZI (mm±SD)**	**±95% CI**	**Standard range(mm)**
E.coli	APF-Ethiopia	4	34±7.1	6.9	30-40
	Brawn Lab.-India	1	32±0	0	
	Cadila Pharmaceutical- Ethiopia	4	34±2.8	2.8	
	EPHARM-Ethiopia	6	32.5±4.6	3.7	
	Huonsco Ltd- S.korea	10	31.1±1.9	1.2	
	Leben lab.-India	7	26.3±8.2	6.0	
	SANDOZ aNavartis-Romania	10	29.1±3.5	2.1	

Among different ciprofloxacillin antibiotics assessed for efficacious test with different manufacturer and brand name, 66.7% of them passed efficacious test as seen in Table 7 below. The highest efficacious test passed observed in CADILA Pharmaceutical- Ethiopia with Ciprodac 500 brand and BRAWN Laboratory-India ciprofloxacillin manufacturers. However, the highest ciprofloxacillin antibiotics efficacious test failure observed in SANDOZ aNavartis-Romania

(70%) and Leben Lab-India (57.1%) with brand name Serviflox 500 and Ciproleb 500, respectively.

Table 7. Efficacious status of ciprofloxacillin antibiotics based on their manufacturers and brand name in Adama city,2018.

Efficacious status of Ciprofloxacillin								
Manufacturer	**country**	**Brand name**	**Conc.**	**Pass**		**Fail**		**Grand Total**
				n	**percent**		**percent**	
APF	Ethiopia	CIF LOX	500mg	3	75	1	25	4
BRAWN Laboratory	India	Brucipro	500mg	1	100	0	0	1
CADILA Pharmaceutical	Ethiopia	Ciprodac	500mg	4	100	0	0	4
EPHARM	Ethiopia	Ciprofloxacin	500mg	5	83.3	1	16.7	6
Huonsco Ltd	S.korea	Floxine	500mg	9	90	1	10	10
Leben laboratory	India	Ciproleb	500mg	3	42.9	4	57.1	7
SANDOZ aNavartis comp	Romania	Serviflox	500mg	3	30	7	70	10
Grand Total				**28**	**66.7**	**14**	**33.3**	**42**

4.3.3. Azithromycine efficacious test

As shown in Table 8, azithromycine manufactured by Sherya life Science-India and Corporation Limited-Kenya had mean inhibition zone of 20±0, Deva Holding-Turkey has mean inhibition zone of 119.33 ± 4.18, Cadila Pharmaceutical-Ethiopia has mean inhibition zone of 18.5 ± 3.5 and Sandoz –Romania has mean inhibition zone of 17 ± 4.2 against *S. aureus.* The minimum mean inhibition zone was 12± 0 manufactured by Bafna Pharmaceutical-India.

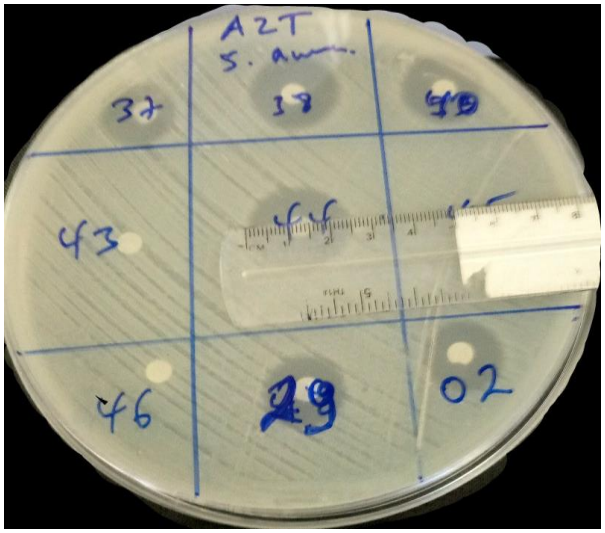

Figure 5. Zone of growth inhibition observed by azithromycine by using strain of *S. aureus* ATCC25923.

The above figure indicates that the azithromycin inhibition zone measurement and the code label on each discs substitute the name of the pharmacy where the antibiotics were collected

Table 8. Mean inhibition zones of azithromycine obtained from various manufacturers in Adama city on *S. aureus* ATCC-25923 organism,2018.

ATTC organism	**Azithromycine Manufacture**	**n**	**Mean ZI (mm±SD)**	**±95 % CI**	**Standard range**
S.aureus	Bafna Pharmaceutical-India	1	12±0.0	0.0	21-26
	Beximico pharmaceutical -Bangladesh	7	16.5±4.6	3.8	
	CADILA Pharmaceutical -Ethiopia	9	18.5±3.5	2.6	
	Coral Lab.-India	2	15.5±3.0	4.2	
	Corporation limited-Kenya	1	20±0.0	0.0	
	Deva Holding-Turkey	5	19.3±4.2	4.4	
	EPHARM-Ethiopia	1	14±0.0	0.0	
	Sandoz –Romania	2	17±4.2	5.9	
	SHERYA LIFE Science- India	1	20±0.0	0.0	
	Umedica Laboratory-India	1	14±0.0	0	
	ZIM lab.-India	8	16.1±3.5	2.98	

Azithromycin purchased from Adama city for efficacious test, 80% manufactured by Deva Holding-Turkey, 42.9% manufactured by Beximico Pharmaceutical-Bangladesh, 44.4% manufactured by CADILA Pharmaceutical-Ethiopia, and 12.5% manufactured by ZIM Laboratory-India were passed efficacious test. More than two-third (68.4%) of azithromycine manufactured by different manufacturers were not satisfactory for efficacious test (Table 9).

Table 9. Efficacious statuses of Azithromycine antibiotics based on their manufacturers and brand name in Adama city, 2018.

Efficacious status of Azithromycin								
Manufacturer	**Country**	**Brand name**	**Conc**	**Pass**		**Fail**		**Grand Total**
				n	**Percent**	**n**	**percent**	
Bafna Pharmaceutical	India	AZIBIAL	500mg	0	0	1	100	1
Beximico pharm	Bangladesh	Azithrocin	500mg	3	42.9	4	57.1	7
Cadila Pharmaca	Ethiopia	Zycin	500mg	4	44.4	5	55.6	9
Coral Lab.	India	Cortzite	500mg	0	0	2	100	2
Corporation lim.	Kenya	THROZA	500mg	0	0	1	100	1
Deva Holding	Turkey	Azitro	500mg	4	80	1	20	5
EPHARM	Ethiopia	Ephazit	500mg	0	0	1	100	1
Sandoz	Romania	Binozyt	500mg	0	0	2	100	2
Sherya life Sc.	India	ZIT	500mg	0	0	1	100	1
Umedica Lab.	India	UZET	500mg	0	0	1	100	1
ZIM laboratory	India	Azito	500mg	1	12.5	7	87.5	8
Grand Total				**12**	**31.6**	**26**	**68.4**	**38**

4.3.4. Ceftraxione efficacious test

As indicated in Table 10, the minimum and maximum mean inhibition zone of ceftriaxone obtained in this study were 32±1.7 and 35±0 manufactured by Gulf pharmaceutical industry-UAE and Vhb medi Science Limited-India respectively. All the mean inhibition zones obtained for

ceftriaxone manufactured by all companies were within the range of the standard(29-35mm) inhibition zone.

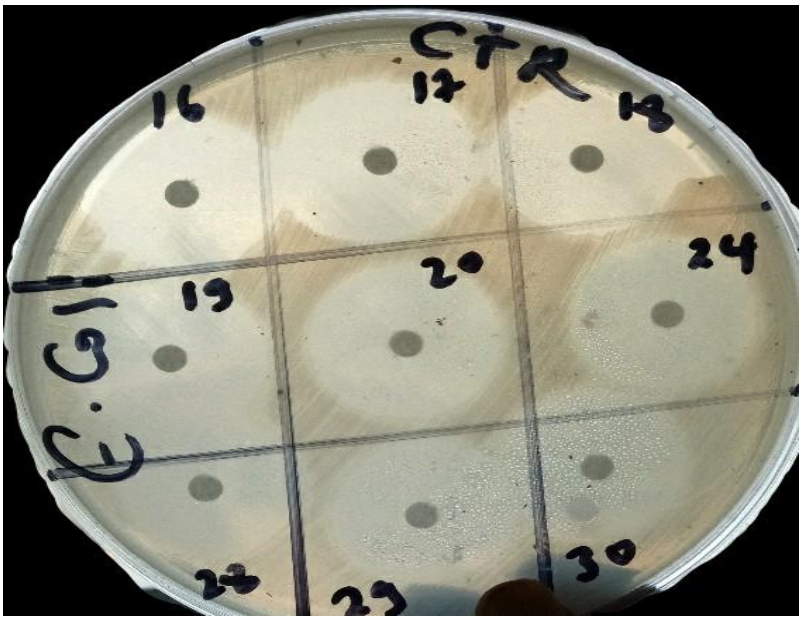

Figure 6. Zone of growth inhibition observed by ceftriaxone by using strain *E. coli* ATCC25922.

From fig. 6, it is clear that ceftraxione inhibition zone measurement and the code label on each discs substitute the name of the pharmacy where the antibiotics were collected

Table 10. Mean inhibition zones of ceftriaxone obtained from various manufacturers in Adama city on *E. coli* ATCC-25922 organism,2018.

ATTC organism	**Ceftriaxone Manufacturer**	**n**	**Mean ZI (mm±SD)**	**±95% CI**	**Standard range(mm)**
E.coli	Ashish life science-India	6	33±1.7	1.3	29-35
	Asral steril Tech-India	5	32.4±1.3	1.2	
	BILIM pharmaceutical- Turkey	2	34±1.4	1.9	
	CSPC Zhougnuo Pharmaceut-China	9	33.2±1.4	0.9	
	Gulf pharmaceutical industry-UAE	3	32±1.7	1.9	
	Shandonglukang Pharmaceut-China	13	32.5±3	1.6	
	Theon Pharmaceutical-India	2	32±1.4	1.9	
	VHB MEDI Science limited-India	1	35±0	0	
	Zhuhai Kinhoo Pharmaceut- China	1	33±0	0	

As indicated in Table 11, all ceftriaxone samples with different manufacturer and brand name purchased for efficacious test from private and governmental pharmacies found in Adama city were passed the efficacious test.

Table 11. Efficacious status of ceftriaxone antibiotics based on their manufacturers and brand name in Adama city,2018.

Efficacious status of Ceftriaxone						
Manufacturer	**country**	**Brand name**	**Conc.**	**Pass**	**Fail**	**Grand Total**
Ashish life science	India	CEFTASH	1000mg	6	0	6
Asral steril Tech	India	ZEFONE100	1000mg	5	0	5
BILIM pharmaceutical	Turkey	FORSEF	1000mg	2	0	2
CSPC Zhougnuo Pharmaceutical	China	Ceftazone	1000mg	9	0	9
Gulf pharmaceutical industry	UAE	Triaxon	1000mg	3	0	3
Shandonglukang pharmaceutical	China	Ceftriaxone	1000mg	13	0	13
Theon Pharmaceutical	India	THEOXONE	1000mg	2	0	2
VHB MEDI Science limited	India	IVIXONE	1000mg	1	0	1
Zhuhai Kinhoo pharmaceutical	China	Ceftriaxone	1000mg	1	0	1
Grand Total				**42**		**42**

4.3.5. Proportion of Efficacious status of all antibiotics

Figure 7, shows proportion of the efficacious status of antibiotics targeted in this study the highest efficacy passed observed in ceftriaxone 100% followed by ciprofloxacillin (69.05%), amoxicillin (52.38%) and azithromycin (31.6%).

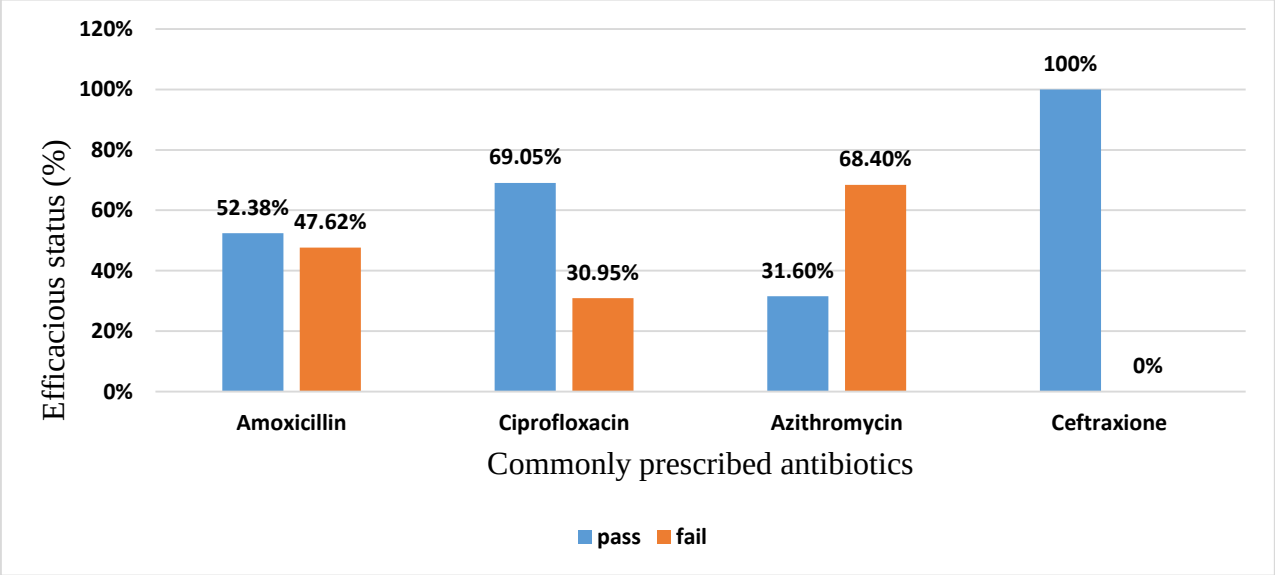

Figure 7. Proportion of efficacious status of commonly prescribed antibiotics based on the standard inhibition zone.

4.3.6. Antibiotics efficacious Status based on country source

During the study period antibiotics imported from Germany, china and UAE passed 100% efficacious test whereas antibiotics imported from South Korea, Turkey, United Kingdom and India passed 90%, 85.7%, 66.7% and 54.8% efficacious test respectively. From locally available antibiotic 54.8 % were passed for antibiotic efficacious test as shown in figure 8.

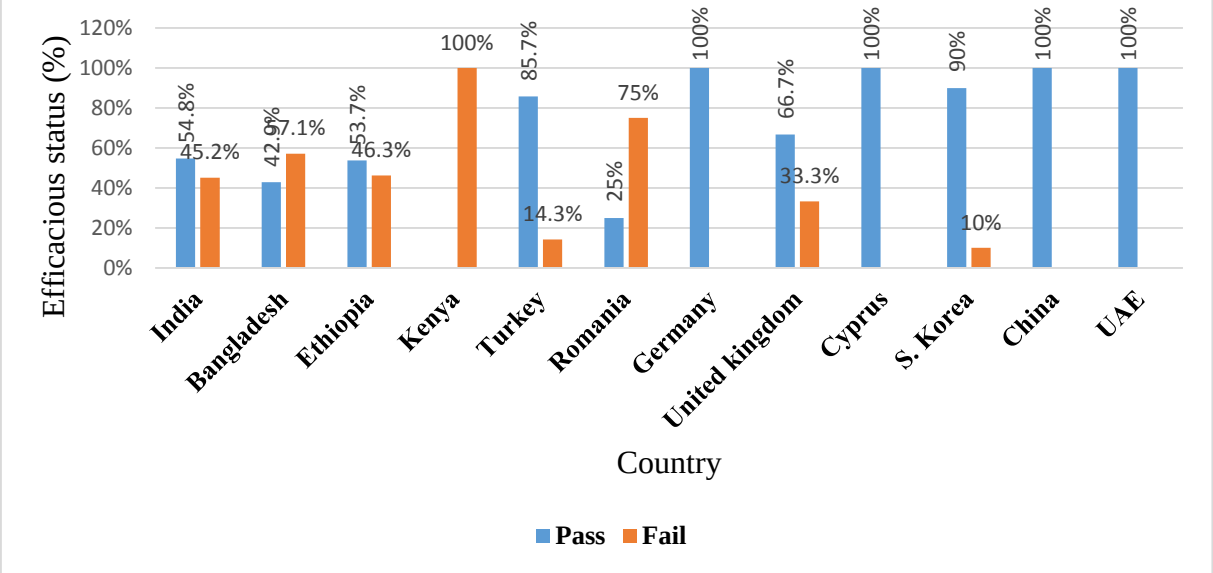

Figure 8. Antibiotics efficacious Status based on country source in Adama city,2018

4.3. Awareness on counterfeit antibiotics and pharmacy monitoring system

Majority (92.9%) of the respondents have no awareness about counterfeit antibiotics and 52.4% of them did not know about individuals who sales unregistered antibiotics. Majority (90.5%) of the respondents replied that there is regular inspection of pharmacies by the responsible regulatory bodies. Of these, 73.8% responded that inspection was in three months and 16.7% of them knew that inspection is in six month intervals (Table 12).

Table 12. Awareness on counterfeit antibiotics and pharmacy monitoring system (n=42)

Variables	Response	frequency	Percent
Awareness on counterfeit antibiotics	Yes	3	7.1
	No	39	92.9
	Total	**42**	**100.0**
Awareness on counterfeit antibiotics	Yes	3	7.1
	No	39	92.9
	Total	**42**	**100.0**
Regulatory body monitoring	Yes	38	90.5
	No	4	9.5
	Total	**42**	**100.0**
Regularity of monitoring	I do not know	4	9.5
	quarterly	31	73.8
	Six month intervals	7	16.7
	Total	**42**	**100.0**

4.4. Shelf life of antibiotics

As indicated in Table 13, amoxicillin and ciprofloxacillin purchased from the private and governmental pharmacies for this study had 100% long expired date (greater than six months). Whereas ceftriaxone and azithromycin 90.5% and 79.5% had long expiry date respectively.

Table 13. Shelf life frequency of most commonly prescribed antibiotics in Adama city (n=164),2018.

Antibiotic	Expiry status	Frequency	percent
Amoxicillin	Expired	0	0
	Long expired date	42	100
	Near Expiry date(<6months)	0	0
	Total	**42**	**100.0**
Ciprofloxacillin	Expired	0	0
	Long expired date	42	100
	Near expiry date(<6months)	0	0
	Total	**42**	**100.0**
Azithromaycin	Expired	0	0
	Long expired date	30	78.9
	Near expiry date(<6months)	8	21.1
	Total	**38**	**100.0**
Ceftraxione	Expired	0	0
	Long expired date	38	90.5
	Near expiry date(<6months)	4	9.5
	Total	**42**	**100.0**

4.5. Storage or handling condition of antibiotics

As shown in Table 14, 59.5% of pharmacies found in Adama city have ventilators with good working conditions, 95.2% of them have large enough storage area, 76.2% have refrigerator and almost 61.9 % and 59.5% of them have thermometer in dispenser and store room respectively.

Table 14. Handling condition of most commonly prescribed antibiotics in Adama city (n=164),2018.

Handling condition	observation	Frequency	Percent
Ventilator	yes	25	59.5
	No	17	40.5
	Total	42	100.0
Large enough storage area	yes	40	95.2
	No	2	4.8
	Total	42	100.0
Refrigerator	yes	32	76.2
	No	10	23.8
	Total	42	100.0
Thermometer in dispenser room	yes	26	61.9
	No	16	38.1
	Total	42	100.0
Thermometer in store room	Yes	25	59.5
	No	17	40.5
	Total	42	100.0

4.6. Demographic result

In this study 42 pharmacies were assessed and among these 34 (81%) were private and 8 (19%) were governmental. Gender, Age category, qualification and work experience of employees is shown in Table 15.

Table 15. Demographic characteristics of dispensers working in private and governmental pharmacies of Adama city (n=42*).*

Variables	Variables category	frequency	percent
Pharmacy ownership	Private	34	81.0
	Government	8	19.0
	Total	**42**	**100.0**
Gender	Male	13	31.0
	Female	29	69.0
	Total	**42**	**100.0**
Age category	≤25years	4	9.5
	26-30years	15	35.7
	31-40years	19	45.2
	41-49yearrs	4	9.5
	≥50years	0	0
	Total	**42**	**100.0**
Qualification of dispensers	Druggist	8	19.0
	Pharmacist	34	81.0
	Total	**42**	**100.0**
Work experience of dispensers	1-4yrs	6	14.3
	5-9yrs	23	54.8
	10-14yrs	9	21.4
	≥15yrs	4	9.5
	Total	**42**	**100.0**

4.7. Factors affecting antibiotic efficacies

As shown in Table 16, the contributing factors considered for antibiotics inefficacious evaluation were pharmacy ownership, air conditioning, shelf life, source of antibiotics, awareness of unlicensed individuals who involved on antibiotic distributing and work experience of the pharmacist or druggist. When we observe antibiotics as a whole there is no significance difference in antibiotic efficacies with in the respective categorical variables. However, with each antibiotic there is significant association between local source and inefficacious status of amoxicillin (x^2=4.7, p< 0.03). There was also statistically significant association between pharmacy ownership and efficacious status of ciprofloxacillin (x^2= 7.4, p< 0. 007).

Table 16. Factors affecting antibiotics efficacies in Adama city,2018.

Variables	Factors	Efficacious status		Chi-square value	Crude Odd Ratio	P-value (P<0.05)
		Pass	Fail			
Pharmacy owner ship	Private	82	54	3.343	2.415	0.067
	Government	22	6			
Amoxicillin	Private	18	16	0.242	0.675	0.623
	Government	5	3			
Ciprofloxacillin	Private	20	14	7.398	0.588	0.007
	Government	8	0			
Azithromycine	Private	11	23	0.930	1.435	0.760
	Government	1	3			
Air conditioning	Ventilation	64	33	0.673	1.309	0.412
	No ventilation	40	27			
Amoxicillin	Ventilation	17	8	4.425	3.896	0.035
	No ventilation	6	11			
Ciprofloxacillin	Ventilation	16	9	0.199	0.741	0.655
	No ventilation	12	5			
Azithromycine	Ventilation	7	15	0.001	1.027	0.970
	No ventilation	5	11			
Shelf life	Long Expired	97	55	0.142	0.794	0.707
	Near expired (<6months)	7	5			
Azithromycine	Long Expired	9	21	0.161	0.714	0.688
	Near expired (<6months)	3	5			
Source of Antibiotics	Local	29	25	3.272	1.847	0.070
	Foreign	75	35			
Amoxicillin	Local	14	17	4.727	0.183	0.030
	Foreign	9	2			
Ciprofloxacillin	Local	11	2	2.969	3.882	0.85
	Foreign	17	12			
Azithromycine	Local	4	6	1.667	2.571	0.510
	Foreign	8	20			
Awareness on unlicensed antibiotic trader	Yes	42	37	0.0012	1.039	0.9726
	No	46	39			
Work experience (handling)	< 10years	76	42	1.879	1.125	0.758
	≥10 years	28	18			

5. DISCUSSION

This current study tried to evaluate the effectiveness of commonly prescribed antibiotics sold in Adama city. The finding in this study revealed that the minimum and maximum mean inhibition zone of amoxicillin were 11.88 ± 4.7 and 21.33 ±2.3, respectively which agrees with findings of 16 ± 0.6 studies done in Ghana, Accra (Helegbe *et al*., 2009). The inhibition zone out range in these samples might be attributed to the inadequate amount of amoxicillin initially(counterfeit), hydrolytic degradation of the amoxicillin due to improper drying, lack of maintaining proper storage condition i.e. amoxicillin should store in temperature between 20-25 ^{o}c but sometimes Adama city may show daily temperature rise of 27-30^{o}c or may be due to the antibiotic manufacturer. The other study done by Idrees (2009) in Subcontinent and the Middle East, showed that out of 11 different amoxicillin samples, 10 samples did not show any counterfeiting and contained amoxicillin more or less the amount claimed by the manufacturers which is disagree with result finding in this studies, among amoxicillin antibiotics 47.6%(20/42) are fail, and only52.4%(22/42) are pass. This difference in inhibition zone is due to many factors, the first is the method used to analysis of efficacy of amoxicillin antibiotics. Second, the quality of antibiotic manufacturer and any other unapparent factors that need more justification. Efficacious antibiotic use has both clinical and economic significance to any health system and should be given adequate attention. Inefficacious use of antibiotics can potentially lead to antimicrobial resistance and increase the necessity to use more expensive antibiotics to treat common and life threatening infections.

In this study the second type of antibiotic used were 500mg ciprofloxacillin which may have prepared in different formulary i.e. 250 mg, 500 mg and 750 mg (ciprofloxacin equivalent) strengths. Reports from the laboratory providing results of the standard single-disk susceptibility test with a 5-µg ciprofloxacin disk should be interpreted according to the CLSI guideline claim that if the antibiotic inhibition zone lies within 30-40 mm diameter range are acceptable (CLSI M100s, 2016). However, in this study, the minimum and the maximum inhibition zone diameter of different brands of ciprofloxacillin were 26.3±8.2mm and 34 ± 7.1mm. Based on the standard claim, 33.3% of ciprofloxacin were out of range and from this failed antibiotics, 50% of ciprofloxacillin sourced from- SANDOZ aNavartis comp in Romania.

The third type of antibiotic was azithromycine, its mechanism of antibacterial activity is by binding to the 50S ribosomal subunit that result in interfering of protein synthesis of the susceptible microorganisms. In this study,15-µg azithromycin prepared disk of different brands provides the minimum and the maximum inhibition zone diameter of 12±0 mm and 20 ± 0 mm respectively using a laboratory test quality control strains: *Staphylococcus aureus* ATCC 25923. It was unsatisfactory inhibition zone according to the CLSI guideline claim inhibition zone diameter, 21-26 mm (CLSI M100S, 2016). This in *vitro* potency outrange inhibition zone finding may be due to the antibiotic azithromycine is markedly affected by the pH of the microbiological growth medium and its dissolubility in 95% ethanol alcohol diluent (Pfizer, 2003).

The fourth antibiotics that used in this antibiotics efficacious test was ceftraxione. The bactericidal activity of ceftriaxone results from inhibition of cell wall synthesis. It has a high degree of stability in the presence of beta-lactamases, both penicillinases and cephalosporinases enzyme of gram-negative and gram-positive bacteria. Ceftriaxone is a compound that has potent bactericidal activity against both gram-negative and gram-positive bacteria (Schmidt et al., 2009). In this research finding, the minimum and the maximum inhibition zone diameter of different brands of ceftriaxone were 32±1.4 mm and 35 ± 0 mm which lies between the manufacturer claim of 29 – 35mm zone diameters by using ATCC Organism E. coli (CLSI M100s, 2016). According to CLSI standard inhibition zone range almost all retailed ceftriaxone antibiotics are 100 % efficacious. In this study the highest efficacy failures observed in azithromycin (68.4%) which is followed by amoxicillin (47.62%) and ciprofloxacillin (30.95%).

The effectiveness of antibiotics can be threatened by external environmental factors such as storage temperature conditions, moisture (humidity) and exposure to light. These environmental factors may negatively affect the chemical composition of antibiotics which in turn affects its potency. when the potency of antibiotic is altered, its expected outcome is put to stop being unable to cure the supposed disease (Wondimeneh et al., 2014). Some of the major concerns expressed by dispensers with regard to antibiotics stability are the effects of the environmental stresses to which antibiotics are exposed throughout a product's lifetime and the effects of such exposure on a product's integrity (USP, 2004). This study showed that 95% of pharmacies found in Adama city have large enough storage area, 59.5% of them have ventilators with good working conditions, 76.2% of them have refrigerator and almost 61.9 % and 59.52% of them have thermometer in dispenser and store room respectively. Based on the standards ventilators,

refrigerator and thermometer expected to have in all pharmacies however, in this research finding there is an indication of poor antibiotic handling in the city. Generally, it is thought that a dispenser should have sufficient knowledge to store and handle antibiotics in a range of room temperature and should have appropriate skills to manage it if fluctuation occur (Hafeez *et al*., 2004).

Expansion of pharmaceutical industries in many countries with advancement in transport technologies facilitated not only trade of genuine pharmaceutical products but also the circulation of poor quality and counterfeited (Kelesidis et al., 2007). Adama city is situated along the road that connects Addis Ababa with Dire Dawa to Djibouti. A large number of trucks and train use this route to travel to and from the seaports of Djibouti. As a result, this makes Adama city one of the busy place in the country with strong business activities and many kinds of illegal commodities including counterfeit antibiotics might be sold in private and government pharmacies found in Adama city and distributed to consumers. So dispensers expect to have information about counterfeit and substandard antibiotics since the safety and efficacy of treatments may be compromised by the availability of counterfeit antibiotics which could have serious consequences for public health (Newton et al., 2006). According to this study, 92.9 % of the respondents had no awareness about counterfeit antibiotics and they did not report to the regulatory body and around 52.4% of them have no information about individuals who participate in pharmacy business unlawfully. However, respondents had relatively better awareness regarding individuals who work pharmaceutical activities illegally than counterfeit antibiotics.

In Ethiopia, Food, Medicine and Health Care Administration and Control Authority was used to regulate both food, health care personnel and the facilities where they were practiced. The mere existence of this legal framework does not guarantee complete absence of illegal, substandard and falsified products as well as illegal establishments in the pharmaceutical chain unless and otherwise pharmacist and druggist pay attention for such case (Sultan *et al*., 2016). Regarding the knowledge of government regulations this study shows that most respondents 90.5% replay that quarterly (73.8%) monitoring of pharmacy occur by regulatory body.

Regarding to the shelf life of antibiotics, 100% of amoxicillin and ciprofloxacillin as well as 90.5% of ceftriaxone and 79.5% of azithromycin that were assessed for efficacy test from the private and Governmental pharmacies have long expiry date (greater than six months). Antibiotics expiration dates are meant to indicate the date at which the antibiotic's potency begins to

diminish. Any antibiotics that contains an organic compound is also susceptible to decay. All antibiotics should be checked for expiry dates and must not be supplied after its expiry date. Factors that will shorten the expiry date of antibiotics are moisture, increased temperature, manufacturing impurities and light specially temperature has a pronounced effect on the rate of degradation of the active ingredient and the rate of decomposition usually doubles for every 10^0C rise in temperature. So pharmacist should observe and adjust the storage conditions specified on the container to increase the long livety of the antibiotics (Stanton *et al.*, 2012).

In Adama city 81% of the pharmacies were owned by private sector and 19% of them were held by government. These pharmacies are managed by a diversity of dispensers in terms of their qualification, age and experience. Most (81%) of the respondents had a university degree (degree of B. pharm) on pharmacy and 19% had level-4 which are druggist with 1-15 years' work experience and majority of them (80.9%) had age 26-49. Gender wise 69% of the respondents were female and 31% were male and this higher percentage of female dispensers were nearly the same with research reports on practice of pharmacist in the European region 63% female and 37% male (Hassel, 2006).

From this study, the contribute factors for failure of commonly prescribed antibiotics were assessed. Based on the assessment result finding, there is no statically association between contributing factors and efficacious of commonly prescribed antibiotics as a whole (Table17). However, regarding to amoxicillin majority of local source were failed with x^2=4.7, p< 0.03. Locally produced amoxicillin antibiotics are more inefficacious than the foreign imported one. The other contributing factors was the presence and absence of air conditioner which has also effect on amoxicillin efficacious status with x^2=4.425, p< 0.035. In addition, 41.2% of the private owned ciprofloxacillin were failed and there is also association between pharmacy ownership and efficacious of ciprofloxacillin with x^2= 7.4, p< 0. 007. Regarding to azithromycine efficacious status test showed that 68.4 % field, however there no statistically relationship with the above listed contributing factors.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

This study concluded that antibiotics produced by different manufacture with different brand name showed different efficacious status. The *in vitro* susceptibility test indicate that all ceftraxione antibiotics were efficacious than ciprofloxacillin, amoxicillin and azithromycine antibiotics. The study showed that there was a problem of poor handling of antibiotics, of which 5% of pharmacies found in Adama city have no large enough storage area, 40.5% of them have no ventilators, 23.8% of them have no refrigerator and almost 38.1 % and 40.48% of them have no thermometer in dispenser and store room respectively. There was significant association between local source and inefficacious status of amoxicillin (x^2=4.7, p< 0.03) and the other contributing factors were the presence and absence of air conditioner which had also effect on amoxicillin efficacious status with x^2=4.425, p< 0.035. There was also statistically significant association between pharmacy ownership and efficacious status of ciprofloxacillin (x^2= 7.4, p< 0. 007).

6.2. Recommendation

Based on the findings of the study, the following recommendations are forwarded.

- Government should initiate surveillance of antibiotics efficacious test to strengthen the production of good quality antibiotics
- Regulatory bodies should monitor antibiotics quality at dispensing area and at production site regularly.
- Regulatory bodies should be reinforced and capacitated in order to address proper post marketing surveillance of antibiotics.
- Further studies shall be done on quantitative based antibiotics studies.

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8. ANNEX

I. Annex

Participants Consent Form

You are being asked to take part in a research study that is screening the effectiveness of commonly retailed antibiotics in government and private pharmacies found in Adama city. You are being asked to be in this study because you are a medical staff member at Adama city, where this study is taking place.

Cost to Subject

You will not be paid for participation in this study.

Voluntary Participation and Study Withdrawal

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you do not take part in the study, you will not lose any benefits.

Invitation for Questions

The researcher carrying out this study is Mr. Demelash Demissie. You may ask any questions you have now. If you have questions later, you may call 0911803697 or 0921092679 at any time.

Confidentiality

your interview records kept confidentially, but it cannot be guaranteed. Records that identify you and the consent form signed by you, may be looked at by the following people:

- Federal agencies that oversee human subject research.
- Institutional Review Board.
- The investigator and research team for this study.
- The sponsor or an agent for the sponsor.
- Regulatory officials from the institution where the research is being conducted, to ensure compliance with policies or monitor the safety of the study.

The results of this research may be presented at meetings or in published articles. However, your name will be kept private. You will also be asked to sign a separate authorization form.

AUTHORIZATION:

I have read this paper about the study or it was read to me. I understand the possible risk and benefits of this study. I know that being in this study is voluntary. I choose to be in this study. I know I can stop being in this study. I will get a copy of this consent form. (Initial all the previous pages of the consent form).

Interviewee: _____

Signature: _____ Date _____

Interviewed by: _____

Signature: _____ Date _____

II. Annex

Structured Interview and observation Questions

The following interview and observational questions are designed to collect data from pharmacy dispensers found in both Private and Governmental pharmacies that will help to Screening the Effectiveness of Commonly retailed Antibiotics from Government and Private pharmacies found in Adama City.

1. The pharmacy you are working now

   private ☐ Governmental ☐

2. Number of staff in the pharmacy

   Druggist…….

   Pharmacist…….

   Others (specify them) ……

3. Sex of interviewee

   Male ☐ Female ☐

4. Age of interviewee

   a. Under 25 years ☐
   b. 26-30 years ☐
   c. 31-40 years ☐
   d. 41-49 years ☐
   e. 50 years and above ☐

5. Educational level of the interviewee

   Level -4 ☐ Bachelor's degree ☐ Master's degree ☐ Doctorate ☐

6. Work experience of the interviewee

   less than 1 year ☐ 1-4 years ☐ 5-9 years ☐ 10-14 years ☐ Above 15 years ☐

7. Which antibiotics are most commonly prescribed by the physicians? Please mention them

   ……

8. Which antibiotics are mostly preferred by the patient?

   Generic ☐ Brand ☐

9. Why do you think they prefer it? ----------

10. Which antibiotics are more found in the pharmacy you dispense?

    Locally produced ☐ foreign imported ☐

11. What counsel you gave for the patient when you dispense antibiotics?

    .....

12. Observe presence of daily temperature record format for

- dispenser rooms: yes ☐ No ☐
- Store room: Yes ☐ No ☐
- refrigerator: Yes ☐ No ☐

13. Observe the air conditioning system in the pharmacy, is it in a good working condition?

    Yes ☐ No ☐

14. Observe Storage area, is the store large enough to allow for order arrangement and proper stock rotation?

    Yes ☐ No ☐

15. Is there any special regulation in antibiotics by regulatory body?

    Yes ☐ No ☐

16. If your answer is yes for the above question how regularly they control?

    ……

17. Do you know authorized or licensed pharmaceutical importers and whole sellers? Please mention some of them……

18. Do you know institutions or individuals involved in pharmaceutical business without being authorized or licensed to provide such services?

    Yes ☐ No ☐

19. What do you think the main contributing factor for the presence of illegal antibiotic sources?

    a.

b. inadequate regulatory measures on illegal institutions or individuals

c. lack of informal market control

d. poor control at entry port

e. poor cooperation between FMHACA and regions

f. extra profit from illegal products

g. All are possible contributing factors

20. What is counterfeit antibiotic?.....

21. Do you have reporting practice about counterfeited antibiotics to regulatory body?

Yes ☐ No ☐

If your answer is no, why? ……..

22. What is Antibiotic resistance? ----------

23. What do you think the main cause of antibiotic resistance?

a. Wrong prescription
b. Substandard antibiotic
c. Miss use of antibiotics
d. Lack of patient counseling
e. Lack of Government commitment to address the issues
f. All are possible causes

Tarree II

Intarviwuu Caaseffamanii fi Gaafilee ilaaluun deebi'an

Intarviwu fi Gaaffileen walitti qabaman kun kan qophaa'an haala daataawwan manneen qoricha dhuunfaa fi Mana qoricha mootummaatti kan argaman calalliidhaa fi dhaabbiidhaan hojji irra oolmaa AntiiBaayootikii Faarmaasiiwwan mana Mootummaa fi Dhuunfaa Magaalaa Adaamaa keessatti .

1, mana qoricha yeroo ammaa itti hojjattu.

A. Kan Dhuunfaa B.kan Mootummaa

2. Saala

A.Dhiira B.Dhalaa

3.Umrii

a.Umrii 25 gadi _____

b.Umrii 26-30 _____

c.Umrii 31-40_____

d,Umrii 41-49_____

e.Umrii 50 fi isaaol kan jiran ____

4.Sadarkaa Barnootaa

Sadarkaa -4_____

Baachulaar Digrii_____

Maastarsi Digrii_____

Dooktireetii _____

5.Muuxannoo Hojii

Waggaa tokkoo gadi_____

Waggaa 1-4_____

Waggaa 5-9_____

Waggaa 10-14_____

Waggaa 15 oli_____

6.AntiiBaayootikii kamtu yeroo hedduu Ogeessa Fayyaan ajajama? Maqaa isaa niieeraa

_____

7.Qoricha kamtu yaalamtootaan irra caalaa filatama?

A, Jeeneerikii B, Biraandii

8.Maaliif waanfilatan sitti fakkaata?_____.

9.Antiibaayootiki iisa kam baay'innaan Faarmaasii kee keessaatti argama?

A,Biyyakeessatti kan Oomishame B, kan biyya alaatii dhufe

10. AntiiBaayotiksi ittifayyadamuu ilaalchisee yaalamtoota maal gorsiteen?

_____

11.GalmeeTeempireecharaa guyyaa guyyaan galmaa'e yoo ilaalamu.

- Kutaa qorichaa Kan itti gurguramu : A,Eeyyen B,Lakki
- Kuusaa Qorichaa: A,Eeyyen B,Lakki
- Rifirijereetara : A, Eeyyen B, Lakki

12.Haala Qileensa Faarmaasii keessa jiru yeroo laaltu.Haala gaarii keessa jiraa?

A,Eeyyen. B,Lakki.

13.Naannoo kuusaa qoricha yeroo laaltu,kuusaa gahaa/bakka gahaa qabaa?

A, Eeyyen B,Lakki

14.Too'annoon addaa AntiiBaayootikii irratti qaama dhimmi ilaallatuun taasifamu nijiraa ?

A, Eeyyen B,Lakki

15.Gaaffiiarmaanolittidhiyaateefdeebiinkee,Eeyyankanjedhuyoota'eakkamiintoo'atu?

_____

16.Impoortaroonni garabiyyaatti qoricha galchan qaama seerawummaa/laayisansiiqabanii fi daldaltoota qoricha raabsan ni beektaa

?Yoonibeektata'eisabeektueerri_____

17.Dhaabbata/namadhuunfaa/seeraan ala ykn laayisansii osoo hinqabaatin biizinasa Rabsa Qoricha irratti hirmaatuun tajaajila kennu ni beektaa?

A, Eeyyen B,Lakki

18.Maddi seeraan ala argamuu antiibaayootiikii maali jetteeyaadda?

A,Too'annaa gahaa Dhaabbilee/namoota dhuunfaa/ seeraanala hojjatan irratti jiraachuu dhabuu.

B,Gabaa seeraan alaa too'achuudhabuu.

C,keellaa irratti too'annaan laafaata'uu

D,walittidhufeenya laafaa B/E/F Naannoo Oromiyaa fi FMHACA jidduujiru.

E,meeshaalee seeraan alaa irratti bu'aa dabalataa argachuu.

F, Gabaabumatti wantoonni armaan olii kunniin sababata'uu nidanda'u

19.kaawuntarfiit AntiiBaayootikii jechuun maal jechuudha?

_____

20.Kaawuntarfiit AntiiBaayootiki iirratti muuxannoo gabaasaa niqabduu?

A, Eeyyen B, Lakki

21.AntiiBaayootik Riizistaansii jechuun Maal jechuudha?

_____

22.Sababbiin ijoon AntiiBaayootik Riizistaansii maalidha jettee yaadda?

A, Ajaja dogongoraa.

B, AntiiBaayootik Sabistaandaardii ta'e.

C, Ittifayyadama seeraan alaa AntiiBaayootikii.

D, yaalamtootaafgorsagahaatahekennuudhabuu.

E, Dhimmicha furuudhaaf kutannaadhabiinsa mootummaa.

F, Gabaabumatti wantoonni armaan oliikunniin sababata'uu nidanda'u.

III. Annex

BIIROO EEGUMSA FAYYAA OROMIYAA

OROMIA HEALTH BUREAU
የኦሮሚያ ጤና ጥበቃ ቢሮ

Lakk/RefNo/ቁጥር AHON/Kun/1-67/393
Guyyaa /Date/ቀን 6/7/2010

Wajjira Eegumsa Fayyaa Bulchinsa Magaala Adaamaa tiif

Hospitaala Medikaala Kollejji Adaamaa tiif

Adaamaa

Dhimmi: **Xalayaa Deeggarsaa Kennuu ilaala**

Akkuma beekamu Biiroon keenya ogeeyyii, dhaabbilee akkasumas namoota qorannoo gaggeessuuf piroppoozaala dhiyeeffatan piroppoozaala isaanii madaaluun akkanumas iddoo biraatti ilaalchisanii fudhatama argatee (approved) dhiyaateef, piroppoozaala isaanii ilaaludhaan waraqaa deeggarsaa ni-kenna. Haaluma kanaan mata-duree " ***Screening the Effectiveness of Commonly retailed Antibiotics from Government and private pharmacies found in Adama City, Oromia Regional State, Ethiopia*** " kan jedhu keessatti **BarataaDigiri 2ffaa Kan ta'ee Obboo Dammallash Damisee** qorannoo geggeessuuf piroppoozaalii isaanii koreen ***"Health Research Ethical Review Committee"*** Biiroo keenyaatti dhiyeeffataniiru. Haaluma kanaan Koreen "**Health Research Ethical Review Committee**" Biiroo keenyaas piroppoozaala kana ilaaluun mirkaneesse jira.

Haaluma kanaan yeroo amma kana immoo Qorannoo kana Magaala Keessaan fi Hospitaala keessaan keessatti hojjechuuf waan karoorfataaniif isiniis kana yaada keessa galchuudhaan deggarsa barbaachisa isaa akka gootan ibsa **Obboo Dammallash Damisee** wayitii qorannoon kun qaacceffamee xumurame fiiriisaa kooppii tokko tokko **BEFO Kuutaa qorannoo fi Qo'annoof** fi iddoo qorannoon irratti adeemsifameef akka galii godhu mallattoo kiyyaan mirkaneessa.

Nagaa Wajjin

Mihiratuu Taarragany
Sadarkaa Itti-Aanaa Hogganaa Biiroo
Abbaa Adeemsa Hojii Dhimmoota
Kominikeeshinii Mootummaa

Maqaa; **Obboo Dammallash Damisee**
Mallattoo________________
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Waajjira Eegumsa Fayyaa
Magaalaa Adaamaa

Adama City Administration Bulchiinsa
Health offic

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Lakk / RefNo WEF Bmn/2238/2010

Guyyaa / Date 9-7-2010

Koollejji Meedikaala Hospitaala_____tiif

Buufataa fayyaa _____tiif

Kilinika _____tiif

Adaamaa

Dhimmi: - **Xalayaa Deeggersaa** Ilaala.

Akkumaa mata dure irraatti ibsuu yaalametti Biiroon Eegumsaa Fayyaa Oromiyaa xalayaa lakk AHDh/km/1-67/393 guyyaa 6/7/2010 nuf barreessanin **Obbo Dammallash Damisee** qorannoo gagessuuf Piroppoozaala dhiyyefachuu isaan nu beeksisee jira. Haalumaa kanan mata duree **"Screening the Effectiveness of Commonly retailed Antibiotics from Government and private pharmacies found in Adama city, Oromia Regional State, Ethiopia"** jedhurratti qorannoo gageessisuuf piroppoozaaliin isaani koree **"Health Research Ethical review Committee"** BEFO waan eyyameef isinis kanumaa hubachuun degersaa barbachisaa ta'e akka gootanif isiin beeksifina.

Nagaa wajjin

Maarishat La'aka(BSC,MPH)
ማርሻት ላዕከ
Q/Balaa Tasaa Fayyaa Hawasaa
ድንገተኛ የሕሰብ ጤና አስተባባሪ

G/G

Obbo Dammallash Damisee tiif

B/jiraanitti

IV. **Annex**

procedure for antibiotic disc preparation

I Preparation of Mueller-Hinton Agar

Mueller-Hinton agar preparation includes the following steps.

1. Mueller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.
2. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.
3. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
4. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
5. Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
6. A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

II Preparation of dried filter paper discs

1. A Whatman filter paper no. 1 approximately 6 mm in diameter Punched out using puncher
2. Wrap up the punched out filter paper with an aluminum foil
3. Sterilized with a stem under pressure autoclave at 120^{o}c for 15 minute
4. After autoclaving, the sterility of the disk checked by placing two to three disk on a Muller Hinton culture media for 16 to 18 hrs., in addition to this, biological (no color change(purple) indicate well sterilized but change purple to yellow after 18hrs incubation not well sterilized) and chemical autoclave indicator (pale brown to dark brown indicate well sterilized) were used.
5. make the disk ready for use after sterility test passed.

III. Preparation of antibiotic stock solutions

1. Antibiotics may be received as powders, capsule or tablets.

2. It is recommended to obtain pure antibiotics from commercial sources, and not use injectable solutions.
3. Accurately weighed Powders capsule were used and then dissolved in the appropriate diluents and agitated by a vortex mixer. (Table-2) to yield the required concentration, using sterile glassware.

S.no	Antibiotics powder and solution	Solvent Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder	Diluent Finishing diluting the final stock solution as stated below
1	Amoxicillin 500mg	Phosphate buffer PH-6.0, 0.01mol/L	Phosphate buffer PH-6.0, 0.01mol/L
2	Ciprofloxacillin 500mg	water	water
3	Azithromycine 500mg	95% of ethanol or glacial acetic acid(1/2 water, glacial acetic acid not greater than 2.5µl/ml	Broth media
4	Ceftraxione1000mg	Water	water

4. Standard strains of stock cultures should be used to evaluate the antibiotic stock solution.
5. Stock solutions are prepared using the formula

$$V = \frac{P * W}{S}$$

$$C = \frac{W}{V}$$

Where, V= volume in ml required for dilution, W= weight of the antimicrobial to be dissolved in V, C= Final concentration of antibiotics, P=dispensing volume on the disc, S=standard disc concentration based on CLSI 2016

6. Dispenses 5µl of antibiotic solution on the disc and left overnight at room temperature to dry.

Antibiotics	W	V	C=W/V	P	S=p*c
Amoxicillin	500mg	250ml phosphate buffer PH-6	2mg/ml=2µg/ µL	5 µL	$10\mu g$
Ciprofloxacillin	500mg	500ml	1mg/ml=1µg/ µL	5 µL	$5\mu g$
Azithromycin	500mg	161.7ml+5ml of 95% ethanol=166.7ml	2.9mg/ml=2.9µg/ µL	5 µL	$15\mu g$

Ceftraxione	1000mg	166.7ml	5.9mg/ml=5.9µg/ µL	5 µL	$30\mu g$

IV. Inoculum preparation

Direct Colony Suspension Method

1. As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from 18 to 24hour agar plate (a nonselective medium, such as MHA should be used).
2. The suspension was adjusted to match the 0.5 McFarland turbidity standard, using saline.

V. Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
2. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inoculate for streaking plates.

VI. Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface.
2. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should

not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.

3. Dispenses 5µl of antibiotic solution on the disc and dried the disc using incubator
4. The plates were inverted and placed in an incubator set to 37°C within 15 minutes after the discs were applied.

VII. Reading Plates and Interpreting Results

1. After 16 to 18 hours of incubation, each plate was examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which was held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. 24 hours of incubation were required If the test organism is a *Staphylococcus* or *Enterococcus* spp.

2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be sub cultured, re-identified, and retested.

3. The sizes of the zones of inhibition are interpreted by referring to page 156, Tables 4A through Zone Diameter Interpretative Standards Breakpoints of the CLSI M100-S26: Performance Standards for antibiotics Susceptibility Testing:

V. Annex

Average Inhibition zone of commonly retailed antibiotics found in Adama city and its efficacious status.

Code	Azithromycin		Amoxicillin		Ciprofloxacin		Ceftraxione	
	Acceptable range (21-26mm)	Antibiotic Efficacious status	Acceptable range (15-22mm)	Antibiotic Efficacious status	Acceptable range (30-40mm)	Antibiotic Efficacious status	Acceptable range (29-35mm)	Antibiotic Efficacious status
	Mean Measured value(mm)		Mean Measured value(mm)		Mean Measured value(mm)		Mean Measured value(mm)	
1	22	Pass	14	Fail	34	Pass	30	Pass
2	13	Fail	16	Pass	31	Pass	34	Pass
3	22	Pass	15	pass	34	Pass	33	Pass
4	22	Pass	21	Pass	27	Fail	34	Pass
5	13	Fail	10	Fail	21	Fail	30	Pass
6	21	Pass	11	Fail	11	Fail	30	Pass
7	14	Fail	11	Fail	33	Pass	30	Pass
8	13	Fail	11	Fail	30	Pass	34	Pass
9	12	Fail	17	Pass	33	Pass	33	Pass
10	13	Fail	10	Fail	30	Pass	30	Pass
11	19	Fail	10	Fail	32	Pass	31	Pass
12	20	Fail	21	Pass	30	Pass	33	Pass
13	21	Pass	20	Pass	33	Pass	33	Pass
14	20	Fail	20	Pass	33	Pass	34	Pass
15	14	Fail	16	Pass	32	Pass	33	Pass
16	14	Fail	11	Fail	32	Pass	33	Pass
17	22	Pass	16	Pass	33	Pass	33	Pass
18	21	Pass	15	pass	32	Pass	33	Pass
19	14	Fail	14	Fail	32	Pass	33	Pass
20	14	Fail	11	Fail	26	Fail	33	Pass
22	16	Fail	9	Fail	33	Pass	30	Pass
24	22	Pass	14	Fail	28	Fail	34	Pass
28	14	Fail	14	Fail	27	Fail	33	Pass
29	17	Fail	16	Pass	28	Fail	33	Pass
30	14	Fail	24	Pass	26	Fail	33	Pass
31	21	Pass	6	Fail	26	Fail	33	Pass
32	17	Fail	16	Pass	26	Fail	30	Pass
34	22	Pass	18	Pass	26	Fail	30	Pass
35	13	Fail	15	pass	25	Fail	30	Pass
36	23	pass	20	Pass	30	Pass	33	Pass

37	18	Fail	16	Pass	27	Fail	33	Pass
38	22	Pass	9	Fail	30	Pass	35	Pass
39	17	Fail	17	Pass	32	Pass	35	Pass
40	20	Fail	9	Fail	32	Pass	35	Pass
42	14	Fail	15	pass	34	Pass	30	Pass
43			7	Fail	34	Pass	33	Pass
44	23	Pass	14	Fail	40	Pass	34	Pass
45	12	Fail	6	Fail	40	Pass	35	Pass
46			15	pass	38	Pass	34	Pass
47			20	Pass	30	Pass	33	Pass
48			6	Fail	40	Pass	40	Pass
49	15	Fail	17	Pass	34	Pass	35	Pass