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Research Article

Evaluation of the Activities of Commonly Dispensed Antibiotics at Bayero University Kano Health Care Services Unit

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ABSTRACT

There is a growing concern about the effectiveness of antimicrobials in developing countries. Multifaceted factors contribute to the escalation of antibiotic resistance. The counterfeiting of antibiotic drugs is one facet of this. Antibiotics are the most counterfeited medicines worldwide. However, the WHO Committee for the review of Medicines accentuates surveillance checks even after the marketing of drugs or at the dispensing point. This study evaluated the activities of commonly dispensed antibiotics at Bayero University Kano health care service pharmacy unit. Fourteen brands of different antibiotics with the active ingredients amoxicillin, azithromycin, cefixime, ceftriaxone, ofloxacin, ciprofloxacin, and doxycycline were used. The antibiotic susceptibility testing was carried out using the Kirby Bauer disc diffusion method on clinical isolates of *Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae*. Typed strains of *E. coli* (ATCC 25923) and *S. aureus* (ATCC 25922) were also tested. Ciprofloxacin and ofloxacin-containing formulations had efficacy against all the clinical isolates and typed organisms. All organisms except *K. pneumoniae* were sensitive to azithromycin. Ceftriaxone was active only against *E. coli* (both clinical isolate and typed organism). About 71% of the antibiotics tested were not sensitive to the tested organisms. The inactivity observed may be due to the counterfeit or substandard nature of the antibiotics, ineffective storage, or the development of resistance. This necessitates more research to determine the extent of the problem and its causes at a large scale.

Keywords: Antibiotics, Resistance, Bayero University, Susceptibility Test, Counterfeit

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INTRODUCTION

In recent years, there has been an increase in antibacterial resistance, posing a need for the production of new antibiotics (Roca *et al*., 2015). Over the past decade, increased public awareness of drug quality has been assessed in terms of counterfeit and or substandard drugs (HealthTech Report, 2014). The World Health Organisation (WHO) estimated that up to 10.5% of the world's pharmaceutical trade consists of counterfeits with 25% of the drugs being consumed in most developing countries (WHO, 2010). The substandard and counterfeit medicines problem is complex but a critical global health concern (WHO, 2007). The activities of antimicrobials are determinant for treatment, the ability of the antimicrobial to penetrate the microbes and kill/inhibit its growth is essential. This issue has significant health and economic consequences, with reports of deaths in Sub-Saharan Africa as a result of counterfeit and substandard treatments (WHO, 2017). The most common type of substandard/counterfeit antimicrobial drugs have a reduced amount of active

drugs, and the majority of them are manufactured in Southeast Asia and Africa (Kelesidis and Falagas, 2015). The growth of the pharmaceutical industry in numerous nations coupled with advancements in transportation technologies allowed for the trading of authentic pharmaceutical items as well as the spread of subpar and fake antibiotics in low-income nations (Kelesidis and Falagas, 2015). The prevalence of diseases that depend on antibiotic therapy treatment such as pneumonia, tuberculosis and diarrhoeal infections are also affected by the use of low-quality antibiotics (HealthTech Report, 2014). Nigerian drug stores sell drugs beyond the expiry date (Owoseye, 2019). There have been reports of counterfeit intravenous meropenem injection (NAFDAC, 2022). A recall of 42 lots of ceftriaxone for injection due to the presence of foreign particulate matter in reconstituted vials has been reported (FDA, 2019). Also, substandard drugs outside pharmacopoeia specifications with undetectable levels of active ingredients have been reported (Hauk *et al*., 2021). This study aims to evaluate the activity of commonly dispensed antibiotics at the pharmacy unit of Bayero University's Health Service Unit, Kano Nigeria. The data obtained will inform the hospital management of the need to set up effective quality control measures and improve reporting channels.

Materials and Methods

Study Area

The study was conducted at Bayero University Kano Health Services, pharmacy unit Kano state Nigeria with latitude and longitude of 12.0022^0N and 8.5920^0E.

Collection of Antibiotics

Approval was obtained from the office of the Medical Director, to gain access to the drugs dispensed at the pharmacy unit. A total of fourteen (14) antibiotics available at the pharmacy from four classes of antibiotics namely penicillins, fluoroquinolones, cephalosporins, tetracyclines and macrolides were collected.

Collection of Bacteria Isolates

Clinical isolates (*S.aureus, E.coli and K.peumoniae*) were collected from the laboratory unit of Bayero University, Kano (BUK) healthcare service unit. Two reference strains *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were obtained from the pharmaceutical microbiology laboratory, Faculty of Pharmaceutical Sciences, Bayero University Kano.

Identification of Isolates

The isolates were identified using Gram staining and biochemical tests;

Gram staining was done according to the standard techniques (Cheesbrough, 2000), a smear of the culture to be stained was made on a clean glass slide and heat fixed. The fixed smear was covered with crystal violet for 60 seconds. This was rapidly washed with clean water; the water was tipped off and the smear was covered with Lugol's iodine for 60 seconds. This was washed off with clean water and decolourized rapidly with acetone-alcohol and washed immediately. The smear was covered with methyl red for 2 minutes and washed off with clean water. The back of the slide was wiped and placed in a draining rack to air dry. A drop of immersion oil was added, and it was viewed under 100x objective of the microscope, for Gram's reaction. This was repeated for all the swabs collected. Isolates were classified as Gram-positive or Gram-negative.

Citrate Utilization

Simmons citrate agar medium was prepared according to the manufacturer's instructions and was inoculated. The slant was inoculated by touching the tip of a needle to a colony that was 18 hours old and incubated at 35°C for 24 hours. The development of a deep blue colour indicates a positive reaction.

Indole Test

A loopful of organism was inoculated into 5mlpeptone water and incubated for 24 hours at 37°C. Three to four drops of Kovac's reagent was then added into the tubes and shaken gently. A positive result is indicated by the formation of a red colour in the layer above the broth.

Catalase Test

A drop of 3 % hydrogen peroxide solution was placed on a glass slide. A loopful of organism was then transferred from the solid medium with a wire loop and emulsified in the hydrogen peroxide. A positive test is indicated by bubbling and frothing.

Coagulase Slide Test

A drop of physiological saline was dropped on a clean glass slide and a colony picked using a wire loop from the solid medium was emulsified in the saline. A loopful of citrated human plasma was added to the bacterial suspension and mixed using the wire loop. The slide was then held up and tilted back and forth for one minute. A positive test is indicated by the clumping of cells in the mixed suspension.

Methyl Red Test (MR)

Four (4) drops of 0.04 % methyl red was dropped in a dextrose phosphate broth culture of the organism. A positive is indicated by a red colour.

VogesProskauer Test (VP)

Exactly One (1) ml of 10 % potassium hydroxide was dropped in a dextrose phosphate broth culture of the organism. A positive test is indicated by a pink colour.

Oxidase Test

Two (2) drops of 1 % freshly prepared oxidase reagent (Tetramethyl-p-phenylenediamine dichloride) was placed on a filter paper in a clean Petri dish. The test organism was smeared on it with a glass rod. A positive result shows a deep purple colour appearing within 5-30secs. The absence of a deep purple colour indicates a negative result.

Urease Test

Christensens urea medium was prepared according to the manufacturer's instructions and was inoculated**.** The slant was incubated at 37°C for 24 hours. The development of a deep red colour with ammonia fumes indicates a positive reaction.

Preparation of antibiotic discs for disc diffusion test

To prepare antibiotic discs, the procedure suggested by the Clinical Laboratory Standard Institute (CLSI) was used. Discs with 6 mm diameter were prepared by punching a sheet of Whatman number 1 filter paper (United Kingdom) using a perforator. In obtaining10 µg of amoxicillin disc and 5µg cefixime disc, 500mg of amoxicillin and 400mg of cefixime tablet were each dissolved in 250 ml of phosphate buffer and 5µl of dissolved antibiotics stock solution were impregnated onto the 6 mm sized disc. Ciprofloxacin tablet (500mg) was dissolved in 500 ml of distilled water, and 5µl of dissolved stock antibiotic was impregnated onto the 6 mm sized disc to obtain a 5µg of ciprofloxacin disc. To obtain 30µg of ceftriaxone, 1000 mg of injectable ceftriaxone was dissolved in 166.7ml of distilled water and 5µl of dissolved stock antibiotic was impregnated onto the 6 mm-sized disc. In obtaining 30ug of doxycycline, 500mg of doxycycline was dissolved in 250ml of water, and 5 µL of the dissolved stock antibiotic solution was impregnated onto the 6 mm-sized disc. Azithromycin (15µg) disc was prepared by dissolving 500mg azithromycin tablet in 166.7 ml of 95% ethanol and 5µl of the dissolved stock antibiotic solution was impregnated onto the 6 mm sized disc. To obtain 5µg of ofloxacin disc, 500mg of ofloxacin tablet was dissolved in 250ml of distilled water and 5mls of 0.1M NaOH was added dropwise until the tablet was dissolved, and the volume made up to 500mls with distilled water, 5µl of the stock antibiotic solution was impregnated onto a 6 mm sized disc (CLSI, 2020)

Standardization of Bacterial Inoculum

Subcultures of clinical isolates (*E.coli*, *S.aureus*, *K.pneumoniae*), *E.coli* (ATCC 25922) and *S.aureus* (ATCC 25923) were made by carefully picking one colony with a sterile inoculation loop, followed by inoculation on the surface of a nutrient agar slant for 24 hours. Using a sterile swab stick, the overnight nutrient broth culture was diluted with saline so that the turbidity was adjusted to the 0.5 McFarland standard which represents 1.5 x 10^{6-8} CFU/ml (CLSI, 2020).

Antimicrobial Susceptibility Testing

Antibiotic susceptibility tests were carried out using the Kirby-Bauer disc diffusion method. Using a sterile dry cotton swab, standardized bacterial suspensions were uniformly inoculated onto the entire surface of Muller Hinton Agar (MHA). Prepared antibiotic discs were placed on the surface of MHA and incubated aerobically at 37°C for 18 hours in an incubator. The diameter of the zone of inhibition was measured using a ruler; isolates were classified as susceptible or resistant based on comparison with the CLSI breakpoint (CLSI, 2020). All experiments were conducted in duplicates and the mean was recorded to determine the susceptibility of the organisms to the antibiotics.

Data Analysis

The activities of each drug on the isolates were expressed as the mean ± standard deviation of n = 14 independent experiments. Statistical significance was considered at $p \leq 0.05$

RESULTS

The antibacterial activity of dispensed medications at the Bayero University Kano health services unit was determined using the disc diffusion method. The values obtained were compared with the values of the Clinical Laboratory Standards Institute (CLSI) for both the clinical isolates and ATCC strains. Table 1 shows the results of the activity of the antibiotics against the clinical isolates.

The diameter of the zone of inhibition of the amoxicillin (10µg) brands tested against *S.aureus* was 7-16.5mm. When compared with the CLSI breakpoint (≥29mm), the brands will be considered to have failed. For *E.coli* and *K. pneumoniae*, the diameter of the zone of inhibition was in the range of 6.5-8.5mm and 7.5-11.0mm. When compared to the CLSI breakpoint (≥17mm), all the tested brands did not have activity against the clinical isolates.

The spectrum of activity of cefixime (5µg) does not cover *S.aureus*, therefore it was not tested against it in this study.For *E.coli* and *K. pneumoniae*, the diameter of the zone of inhibition was 6-11mm while the CLSI breakpoint was≥19. This shows that the brands of cefixime tested did not have activity against the clinical isolates.

The diameter of the zone of inhibition for azithromycin (15µg) against *S. aureus*for the two brands tested was 23-25mm (CLSI ≥18). Therefore they were active against the tested organisms. A similar result was observed with *E.coli*, with diameters of the zone of inhibition ranging from 19.5-20.5mm (CLSI≥13). This shows that the brands tested are active against *E. coli* clinical isolate. For *K. pneumoniae*, brand (f) had a zone of inhibition of 8mm in diameter while brand (g) had a diameter of 14mm (CLSI≥13mm). Therefore, only brand (f) was active against *K. pneumoniae*. The cause for the difference was not further investigated in this study.

Doxycycline (30µg) brands tested were not active against *S.aureus* (6-9mm; CLSI ≥16mm) and *E.coli* (6mm; CLSI≥14mm) but sufficient potency was observed with *K.pneumoniae* (16-30mm; CLSI ≥14)

Ceftriaxone (30µg) was only active against *E.coli* (34mm; CLSI ≥23mm). All ofloxacin (5µg) and ciprofloxacin (5µg) brands tested were active against *S.aureus*(18-19mm; CLSI≥18mm) and (21.5-24mm; CLSI≥21mm) respectively. Similarly, significant activity was observed with ofloxacin and ciprofloxacin brands against *E.coli* with diameters of zone of inhibition ranging from (19.5-31.0mm; CLSI ≥16mm) and 28.5-37mm; CLSI ≥26mm) respectively. However, for *K. pneumoniae*, ofloxacin brand (l) was not active against the clinical isolate. But all the tested ciprofloxacin showed significant activity (36-36.5mm; CLSI 26mm).

The antibacterial activity of tested antibiotics against typed organisms is shown in Table 2 below. *S.aureus*(ATCC 25923) was susceptible to all tested brands of azithromycin (21-26mm; CLSI ≥18mm), ofloxacin (20-25mm; CLSI ≥18mm) and ciprofloxacin (21mm; CLSI ≥21mm). A significant difference in the activity of azithromycin on *S.aureus* (clinical isolate) and the typed organism was observed ($p = 0.027$). However, amoxicillin, doxycycline and ceftriaxone did not pass the susceptibility test with diameters of zone of inhibition ranging from (7-25mm; CLSI ≥29, 6-9mm; CLSI≥16 and 25mm≥29mm) respectively.

The diameters of the zone of inhibition of azithromycin (17-23mm; CLSI≥13), ceftriaxone (35mm; CLSI≥23mm), ofloxacin (34-35mm; CLSI≥16mm) and ciprofloxacin (36-37mm; CLSI≥26mm) showed they were all active against. *coli* (ATCC 25922). There was a significant difference between the efficacy of azithromycin against *E. coli* (clinical isolate) and the reference *E. coli* ($p = 0.016$). However, all tested brands of amoxicillin (7-8mm; CLSI≥17mm) and cefixime (6-11mm; CLSI≥19mm) were not active. Doxycycline brand (h) had a zone of inhibition of 11mm in diameter while brand (i) had a zone of inhibition of 16mm. From the reference CLSI value of ≥14mm, therefore *E. coli* (ATCC 25922) was susceptible to brand (i) but not (h).

Table 1: Antibacterial sensitivity of drugs dispensed at Bayero University Kano Health Services Unit against clinical isolates. Data represent the mean ± standard deviation of n = 14 independent experiments

Antibiotic	Brand name	Disc potency	Diameter of the zone of inhibition (mm)		
			S. aureus	*E. coli*	*K.pneumoniae*
Amoxicillin	Neomixil (a)	10µg	16.5±0.5	6.5±0.1	7.5±1.1
	Olimox (b)		16.0±1.5	8.5±0.5	11.0±1.2
	Annmox(c)		7.0±0.3	7.5±0.5	7.0±0.5
	Standard		≥29	≥17	≥17
Cefixime	Ezcef (d)	5µg	Nil	11.0±0.8	6.0±0.2
	Cefrite(e)			6.0±0.5	6.0±0.1
	Standard			≥19	≥19
Azithromycin	Krishat Azithromycin(f)	15µg	25.0±1.2	19.5±2.8	8.0±0.6
	TGP Azithromycin(g)		23.0±1.5	20.5±3.5	14.0±0.9
	Standard		≥18	≥13	≥13
Doxycycline	Maydon(h)	30µg	9.0±1.8	6.0±0.5	30.0±2.9
	Aldox(i)		6.0±0.8	6.0±0.1	16.0±1.8
	Standard		≥16	≥14	≥14
Ceftriaxone	Zoxon(j)	30µg	12.5±1.1	34±3.5	9.0±0.5
	Standard		≥29	≥23	≥23
Ofloxacin	Kesflox(k)	5µg	18.0±2.6	31.0±2.8	16.0±1.1
	Savoflox(l)		19.0±1.3	19.5±1.2	12.0±1.3
	Standard		≥18	≥16	≥16
Ciprofloxacin	Ultraflox(m)	5µg	21.5±0.1	28.5±2.2	36.0±3.8
	Guciprox(n)		24.0±0.5	37.0±3.5	36.5±4.1
	Standard		≥21	≥26	≥26

Note: mm: millimetre, SD: Standard deviation, µg: microgram, Nil: Cefixime has no activity against *S. aureus*

Table 2: Antibacterial sensitivity of drugs dispensed at Bayero University Kano Health Services Unit against typed organisms. Data represent the mean ± standard deviation of n = 14 independent experiments

Antibiotic	Brand name	Disc potency	Diameter of the zone of inhibition (mm)	
			S. aureus **(ATCC 25923)**	***E. coli*** **(ATCC 25922)**
Amoxicillin	Neomixil (a)	10µg	19±1.8	7±0.1
	Olimox (b)		25±2.6	8±0.5
	Annmox (c)		7±0.1	7±1.6
	Standard		≥29	≥17
Cefixime	Ezcef (d)	5µg	Nil	11±0.2
	Cefrite (e)			6±0.5
	Standard			≥19
Azithromycin	Krishat Azithromycin(f)	15µg	21±2.2	17±2.1
	TGP Azithromycin(g)		26±3.1	23±3.1
	Standard		≥18	≥13
Doxycycline	Maydon(h)	30µg	6±0.1	11±1.1
	Aldox(i)		9±0.2	16±0.9
	Standard		≥16	≥14
Ceftriaxone	Zoxon(j)	30µg	25±2.6	35±3.2
	Standard		≥29	≥23
Ofloxacin	Kesflox(k)	5µg	20±0.6	35±3.0
	Savoflox(l)		25±1.2	34±2.8
	Standard		≥18	≥16
Ciprofloxacin	Ultraflox(m)	5µg	21±1.3	36±3.9
	Guciprox(n)		21±0.9	37±2.7
	Standard		≥21	≥26

Note. mm: millimeter, SD: Standard deviation, µg: microgram, ATCC: American type culture collection, Nil: Cefixime has no activity against *S. aureus*

DISCUSSION

Regarding the counterfeiting of drugs, this study aimed to evaluate the activity of antibiotics dispensed at the Bayero University Kano health services unit, Nigeria. A post-marketing surveillance of essential medications including antibiotics is generally advised by the WHO, even after they have been marketed or are being distributed (Raj *et al.,* 2019). All the brands of amoxicillin and cefixime tested were not active on both clinical isolates and typed strains. A similar result has been reported in Calabar (Nkang*et al*., 2010). This suggests a defect in the antibiotics themselves, their storage, the limitations of our laboratory techniques, or the possibility that the antibiotics are counterfeit or of inferior quality (Taberner*et al*., 2019). Bacteria isolated from clinical specimens were reported to have reduced susceptibility to amoxicillin (Tesema*et al*., 2020; Mohammed *et al*., 2020). A similar study reported that 50% of amoxicillin tested to be inactive against clinical and typed organisms (Demissie*et al*., 2021).

The two brands of azithromycin tested were found to be active against the typed organisms and clinical isolates. However, one brand was not active against *K. pneumonia having* a mean diameter zone of inhibition of 8mm as opposed to the CLSI guideline of ≥13mm. This does not agree with the data of Demissie*et al*., (2021) who reported a 100% lack of potency of azithromycin to *S. aureus* (ATCC 25923).

Doxycycline was active against *K. pneumoniae* and *E. coli* (ATCC 25922). About 100% resistance to doxycycline by *E.coli, S. aureus,* and *K.pneumoniae* has been reported (Okonko*et al*., 2009b). Resistance to tetracycline is common and this has been further confirmed (Adedeji and Abdulkadir, 2009).

Ceftriaxone was only potent against *E. coli* (clinical isolate) and *E.coli* (ATCC 25922). Similar reports of documented lack of susceptibility which is indicative

of resistance have been obtained (Alabi and Ijose2015).

From the two brands of ofloxacin tested, significant activity was observed in both clinical and typed organisms. However, inactivity was observed with one of the brands against *K. pneumoniae*. This is indicative of resistance. Although the natural phenomenon of resistance development is accelerated and magnified by a variety of factors (Eger *et al*., 2022), one of the most important causes is the improper use of antimicrobial agents.

Ciprofloxacin brands tested were active on both clinical isolates and reference used in this study. However, this was not the case in a study carried out in Ethiopia, where a significant lack of potency was reported (Demissie *et al*., 2021).

Some minor variations in the activity of some brands of antibiotics used in this study against the same organism were also observed. This is not addressed further in this study, but it could be due to differences in the formulation methods and the excipients used. This can have a significant effect on the ability of the antibiotics to penetrate the bacterial cell and exert their action. Another possible explanation could be the effect of storage conditions, as this could affect the overall efficacy of the medication (Zilker*et al*., 2019).

The substandard and counterfeit medicines problem is complex but a critical global health concern (WHO, 2007). Epidemiologic surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance control measures and preventing the spread of antimicrobial-resistant microorganisms. The worldwide escalation of both community and hospital-acquired antimicrobial-resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control and new treatment alternatives (Chikere et *al*., 2008). The World Health Organization has recommended post-marketing surveillance of essential drugs, therefore microbiological disc diffusion (Comparative method) was employed which is the most widely used for routine sensitivity testing (WHO, 2021).

The worldwide trend of empirically treating infections may not work well in Nigeria, because decreased susceptibility rates have been documented for the majority of the common pathogens in various parts of the country (Abubakar, 2009)

Conclusion

This study reveals a lack of activity by the majority of the antibiotics tested against typed organisms which is suggestive of counterfeiting or lack of proper storage. Therefore there is a need for further investigation to determine the magnitude of the problem and its cause at a larger scale. RegulatoryAgencies involved in regulating the sale of medicines should apart from ascertaining that the medicines imported into the country are of the required standard, also ensure their storage under appropriate conditions, especially antibiotics, which require strict storage conditions. Moreover, strategies to monitor the efficacy of antibiotic medicines and to eradicate counterfeit or substandard medicines should be intensified.

REFERENCES

AbubakarE.M.M (2009). Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the Specialist Hospital, Yola, Adamawa state, Nigeria. Journal of clinical Medicine and Research. https://doi.org/10.5897/JCMR.9000009

AdedejiB.A.M., AbdulkadirO.A,(2009). Aetiology and Antimicrobial Resistance Pattern of Bacterial Agents of Urinary Tract Infections in Students of Tertiary Institutions in Yola Metropolis. Advanced. Biology. Research. **3**(3-4): 67-70.

Alabi, O.; Ijose, A. (2015) Microbiological evaluation of the potencies of brands of four parenteral antibiotic preparations used in the treatment of urinary tract infections. Afr. J. Clin. Exp. Microbiol. **16**:128–133.

Cheesebrough, M. (2000). *Medical Laboratory Manual for Tropical Countries* vol.II Microbiology Cambridge University Press, U.K. PP. 143-147.

ChikereC.B, ChikereB.O, OmoniV.T (2008). Antibiogram of clinical isolates from a hospital in Nigeria. *African. Journal ofBiotechnology*. **7**(24): 43594363.

Clinical and Laboratory Standards Institute. Performance for antimicrobial disk susceptibility tests; approved standard, CLSI documentM100S. 26th ed. Wayne (PA), USA: Clinical and Laboratory Standards Institute. 2020; **32** (1):156.

Demissie, D., Geremew, T., Chernet, A. Z., & Ali, M. M. (2021). Potency of commonly retailed antibiotics in pharmacies found in Adama, Oromia regional state, Ethiopia. PloS one, **16**(7), e0253971. https://doi.org/10.1371/journal.pone.0253971

Eger, E.; Schwabe, M.; Schulig, L.; Hübner, N.O.; Bohnert, J.A.; Bornscheuer, U.T.; Heiden, S.E.; Müller, J.U.; Adnan, F.; Becker, K. (2022). Extensively Drug-Resistant Klebsiellapneumoniae Counteracts Fitness and Virulence Costs That Accompanied Ceftazidime-Avibactam Resistance Acquisition. Microbiol. Spectr., 10, e00148-22. [CrossRef] [PubMed]

FDA. (2019) Lupin Pharmaceuticals, Issues Voluntary Recall of Ceftriaxone for Injection USP, 250mg, 500mg, 1g and 2g.

Hauk, C., Hagen, N., &Heide, L. (2021). Identification of Substandard and Falsified Medicines: Influence of Different Tolerance Limits and Use of Authenticity Inquiries. The American journal of tropical medicine and hygiene, **104**(5), 1936–1945. https://doi.org/10.4269/ajtmh.20-1612

HealthTech Report (2014). *Prevalence of counterfeit and substandard antibiotics in developing countries*. Seattle, Washington: PATHOLOGY; 2014.

Kelesidis, T., &Falagas, M. E. (2015). Substandard/counterfeit antimicrobial drugs. Clinical microbiology reviews, **28**(2), 443–464. https://doi.org/10.1128/CMR.00072-14

Mohammed, A.A, Ali, M.M., Zenebe, M.H. (2020). Bacterial aetiology of ocular and periocular infections, antimicrobial susceptibility profile and associated factors among patients attending eye unit of Shashemene Comprehensive Specialized Hospital, Shashemene, Ethiopia. BMC Ophthalmology, 20:24. https://doi.org/10.1186/s12886-019-1301-0 PMID: 31931766

NAFDAC (2022). Alert on counterfeit meropenem circulating in Nigeriahttps://www.nafdac.gov.ng/public-alert-no-045-2022-alert-on-counterfeit-meropenem-circulating-in-nigeria/

Nkang A.O, Okonko I. O, Lennox J. A, Eyarefe O. D., Abubakar M. J, Ojezele M. O., .Babalola E. T., Ogunnusi,T, Onajobi. A. I, Amusan T. A(2010) Assessment of the efficacies, potencies and bacteriological qualities of some of the antibiotics sold in Calabar, NigeriaAfrican Journal of Biotechnology Vol. **9**(41), pp. 6987-7002

Okonko I.O., Soleye,F.A., Amusan,T.A., Ogun,A.A., Ogunnusi T.A., Ejembi (2009b). Incidenceof Multi-drug Resistance (MDR) organism in Abeokuta, Southwestern Nigeria. Global Journal of Pharmacology. **3**(2): 69-80.

Owoseye, A. (2019) How Nigerian drug stores sell drugs beyond expiry date.https://www.premiumtimesng.com/author/ayo damola

Raj, N.; Fernandes, S.; Charyulu, N.R.; Dubey, A.; G., S.R.; Hebbar, S.(2019). Postmarket surveillance: A review of key aspects and measures on the effective functioning in the context of the United Kingdom and Canada. Ther. Adv. Drug Saf., 10, 2042098619865413. [CrossRef] [PubMed]

Roca I., Akova M., Baquero F., Carlet J., Cavaleri M., Coenen S., Cohen J. (……), Vila J. (2015). The global threat of antimicrobial resistance: Science for intervention. *New Microbes and New Infections*, 6, pp. 22-29

Tabernero P, Swamidoss I, Mayxay M, Khanthavong M, Phonlavong C, VilayhongCet l.(2019). A random survey of the prevalence of falsified and substandard antibiotics in the Lao PDR. AntimicrobChemother; 74: 2417–2425. https://doi.org/10.1093/jac/dkz164 PMID: 31049576

Tesema NN, Zenebe MH, Ali MM. Bacterial associated urinary tract infection, risk factors and drug susceptibility profile among adult people living with HIV at Hawassa University Comprehensive specialized Hospital, Hawassa, Southern Ethiopia. Nature Scientific Reports. 2020.

WHO. (2007). World Health Organization. *Quality Assurance of Pharmaceuticals. A Compendium of Guidelines and Related Materials*. Geneva, Switzerland:
2007. https://www.who.int/medicines/areas/quality_safety/quality_assurance/Qualitya

WHO. (2010). World Health Organization. *Survey of the Quality of Selected Antibacterial Medicines Circulating in Six Countries of Sub-Saharan Africa*. Geneva, Switzerland: World Health Organization; 2010.

WHO (2017): antimicrobials Resistance and its consequences. Retrieved June 7th 2018. *http//www.int/healthinfo/bodgbd2017/index.html*

WHO. (2021). World Health Organization: Experts caution Nigerians against indiscriminate use of antibiotics to reduce future health crisis; http://www.who.int./antibiotics

Zilker, M.; Sörgel, F.; Holzgrabe, U.(2019) A systematic review of the stability of finished pharmaceutical products and drug substances beyond their labelled expiry dates. J. Pharm. Biomed. Anal, 166, 222–235. [CrossRef] [PubMed]