WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 7, 845-859. Research Article ISSN 2277– 7105

PHYSICOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF ERYTHROMYCIN TABLET USING UV SPECTROSCOPY: A COMPARATIVE STUDY

Ebiere Dode*[1], Baraikio Didi[2] and Josiah G. Puyate[1]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Article Received on
14 March 2023,

Revised on 04 April 2023,
Accepted on 25 April 2023

DOI: 10.20959/wjpr20237-28029

***Corresponding Author**
Ebiere Dode
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

ABSTRACT

Erythromycin, a macrolide antibiotic produced by strain of *Saccharopolyspora erythreas* is effective against gram-positive and gram-negative bacteria by inhibiting protein synthesis. They exist as tablet, capsule, or suspension, injectables, ophthalmic solution and ointments. They are one of the most frequently prescribed second-line antibiotics in Nigeria for the treatment of respiratory tract infections. Due to availability of adulterated and substandard drugs, antimicrobial resistance (AMR) is on the rise, particularly in the sub-Saharan region. Weight uniformity test, disintegration test, dissolution test, UV spectroscopy, and microbiological assay were used to examine five different brands of erythromycin stearate tablets. The weight uniformity test was done using analytical balance to compare their respective weights. The disintegration test was done using disintegration apparatus and distilled water as the medium. The dissolution test was done using dissolution apparatus and 0.1 N HCl as the medium. The microbiological assay was tested with the following bacteria using the Agar well diffusion method: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Both disintegration test and weight uniformity test were passed by all brands. Except for E4, all brands passed dissolution test. All brands had percentage purity levels within the allowed range for the chemical analysis. For the microbiological assay, against *E. coli*, all brands have high antibacterial activity except E3 with intermediate antibacterial activity. Against *S. aureus*, all

brands had intermediate antibacterial activity. Against *P. aeruginosa*, all brands had high antibacterial activity except E4 with intermediate antibacterial activity. Against *B. subtilis*, all brands had high antibacterial activity.

KEYWORD: Erythromycin, AMR, disintegration, dissolution, UV spectroscopy.

INTRODUCTION

The macrolide antibiotic erythromycin was first discovered in 1952 and is made by a strain of Saccharopolyspora erythreas.[1],[2],[3] They are used to treat infections of the skin and soft tissues, and the respiratory system, chlamydia infections, pelvic inflammatory disease, and syphilis.[2],[4] They are effective against both gram-positive and gram-negative bacteria.[2] They are bacteriostatic and prevent the synthesis of proteins by attaching to the human-absent 23S ribosomal RNA molecule in the bacterial ribosome's 50S subunit.[1] Additionally, they have immunomodulatory and anti-inflammatory effects.[1] There are Erythromycin A, B and C of which Erythromycin A is the most common and the IUPAC name is (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione with a molecular weight of 733.937 g/mol^{-1} and molecular formula $C_{37}H_{67}NO_{13}$.[2] Below is the chemical structure.

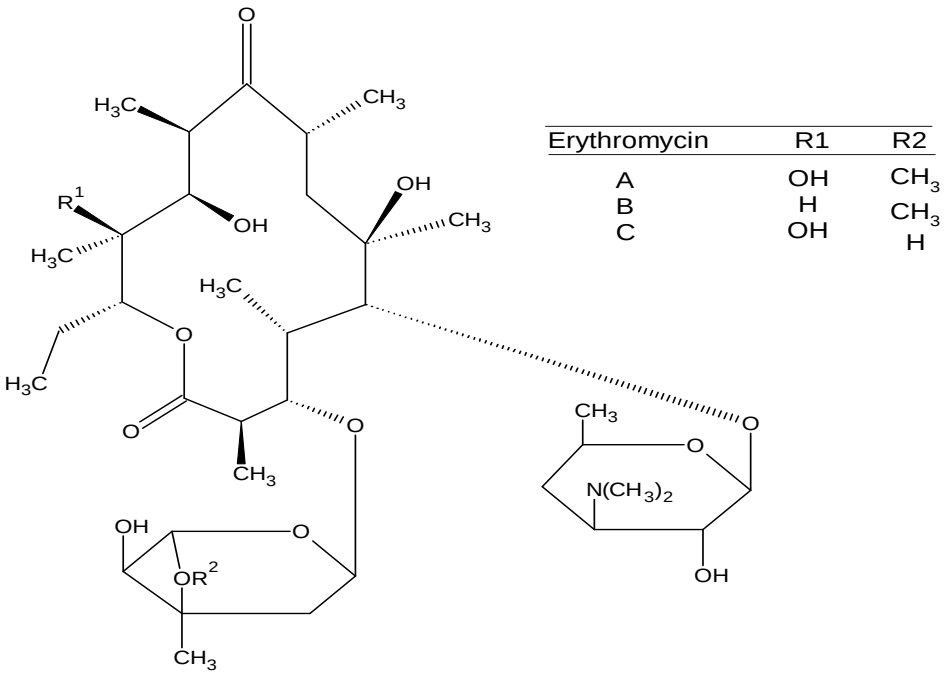

Figure 1: Structure of Erythromycin.

Enteric-coated tablets, slow-release capsules, oral suspensions, ophthalmic solutions, ointments, gels, non-enteric-coated tablets, non-enteric-coated capsules, and injections are all forms of erythromycin that are available.[2] They can be found as salts in various formulations. They may come in the forms of stearate, ethyl succinate, and estolate for tablets and oral suspension. Regarding capsules, it might be estolate or base, and erythromycin base is also present in eye ointment. Gluceptate and lactobionate are the injectable salts.[2]

Antimicrobial resistance (AMR), a global issue that is estimated to be responsible for 700,000 deaths each year, will likely result in the loss of 10 million lives and US $100 trillion annually by 2050 if no appropriate action is taken[5],[6],[7],[8] With a death rate of 27.3 per 100,000 people, Sub-Saharan Africa has the highest AMR mortality.[9] AMR is said to happen when microorganisms like bacteria, fungi, viruses, and parasites change in response to antimicrobial drugs, according to the World Health Organization (WHO).[10] Antibiotics, antifungals, antivirals, antimalarials, and anthelmintics are examples of antimicrobial medications.[10] Drugs lose their effectiveness, infections linger in the body, and the likelihood that they will infect others rises.[10] The likelihood that antibiotics will be able to treat common infectious diseases is decreasing due to the frequent development of new resistance mechanisms by microorganisms, which is leading to prolonged illness, disability, and even death.[10] Major medical procedures, such as organ transplantation, cancer chemotherapy, diabetes management, Caesarean section, etc., may be high risk without effective antimicrobials for the prevention and treatment of infectious diseases.[10] The likelihood of achieving the Sustainable Development Goals and the Millennium Development Goals is therefore low.[10] Although microorganisms can become resistant to antibiotics, our actions can also promote the spread of resistance.[11] These could occur due to overuse of antibiotics by medical professionals, patient non-compliance with prescriptions, poor hygiene, inadequate prevention methods, lack of control, and international travel.[10],[11],[12] Another factor contributing to AMR, particularly in LMIC, is the entry of fake and adulterated medications onto the drug market. Due to the development of drug resistance by the microorganisms, therapeutic success is compromised when the bacteria are exposed to sub-therapeutic doses of the medication.[13],[14],[15] There is also the issue of unregulated supply chains and the accessibility of antimicrobials without a prescription[14],[16], where 80% of those handling the drugs have no knowledge of drug dispensing.[14],[17],[18] High temperatures and humidity in Africa's weather can have an impact on the quality of antimicrobial drugs while they are being stored, in addition to poor storage

conditions.[15],[19],[20] Assay of antibiotics is mainly carried out by microbiological screening however this test lack specificity hence chemical and instrumental methods are used for analysis.[21] For the chemical analysis of erythromycin, a few techniques have been used, including electrochemistry, spectroscopy, and chromatography.[21] A popular technique for analysis by separation from other components and detection by a UV detector is high-performance liquid chromatography (HPLC).[22],[23] Mahmoudi and Boukhechem created a novel HPLC method for erythromycin evaluation in human urine using an end capped ODB RP18 column as the stationary phase and acetonitrile -2-methyl-2-propanol-hydrogenphosphate buffer, pH 6.5, with 1.5 percent triethylamine (33:7: up to 100, v/v/v) as the mobile phase.[24] Erythromycin has been separated from erythromycin stearate tablets and erythromycin estolate suspension using silica gel on Thin Layer Chromatography (TLC).[25],[26] Using a TLC plate coated with Kieselgel GF254, erythromycin A, B, C, and D were separated from one another.[26],[27] Erythromycin has been quantified and separated using gas chromatography using flame-ionization detection in erythromycin tablets,[26],[28] It has also been reported that pyrolysis-gas chromatography can be used to quantify the amount of erythromycin in erythromycin ethyl succinate capsules,[26],[29] With erythromycin dissolved in the running buffer at various concentrations ranging from 0.1 to 10 mM and three different Background Electrolytes (BGEs), sodium phosphate pH 3.0 and 7.0 and sodium borate pH 9.2, the chiral selection of erythromycin has been separated using capillary zone electrophoresis.[30] Erythromycin in pharmaceutical formulations has been examined using direct UV and first derivative measurements at wavelengths of 285 and 300 nm, respectively.[31],[32] Using transmission Fourier Transform Infrared (FT-IR) spectroscopy, a straightforward and economically viable analytical method was developed to quantify erythromycin in tablet formulation.[26],[33] FTIR is used for the quality control analysis of erythromycin and its impurities. It is based on the measurement of an object's infrared radiation transmission or absorption.[33] The chemical makeup of erythromycin and its derivatives can be thoroughly analyzed using nuclear magnetic resonance (NMR) spectroscopy. For instance, the conformational blend of new members of a series of erythromycin A derivatives in Chloroform-D solution was predicted using ^{1}H and ^{13}C NMR chemical shift data.[34] NMR spectroscopy was also used to investigate the acid-catalyzed degradation of clarithromycin and erythromycin B.[35] Other analytical techniques have been used in conjunction with mass spectroscopy (MS). Erythromycin and Tylosin in honey were subjected to quantitative analysis using solid-phase extraction and mass spectrometry.[36] Liquid chromatography analysis using electrospray ionization mass spectrometry was used to

quantitatively analyze erythromycin.[37],[38],[39] For the analysis of erythromycin, electroanalytical techniques such as amperometric[40],[41],[42], voltametric[43],[44], coulometric[45], and potentiometric detection[46] have been used. Comparing the inhibition of microorganism growth caused by known antibiotic concentrations allows us to gauge an antibiotic's potency.[26] Analysis of erythromycin can be carried out by microbiological assay and comparing their respective zone of inhibition diameter to kirby-Bauer (Antibiotic Sensitivity) guideline charts.[47] The use of agar diffusion agar method has been used in the validation of erythromycin microbiological assay.[48],[49]

MATERIAL

Apparatus

UV model Spectrum Lab 752 Pro MMMs Spectrophotometer, Ohaus Adventurer Pro Analytical balance, Veego VDA-6DR USP Standard tablet dissolution apparatus, Veego USP Standard disintegration apparatus, Autoclave, Funnel, Pipette, Beakers, Volumetric flask, Conical flask, filter paper, measuring cylinder, syringe, Mueller Hinton agar, Petri dish,

Reagents

Distilled Water, Methanol, 0.1N HCL.

Micro-organism

Bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*).

Drug sample

Five brands were purchased from retail pharmacy in the state capital, Yenagoa and were coded E1, E2, E3, E4 and E5. All the brands were imported, and none were expired as at when analysis was carried out and only E1 was registered with the drug regulatory body in Nigeria.

METHOD

Weight uniformity test

Twenty tablets of E1 were randomly selected and weighed individually using an analytical balance and their respective weights recorded. The procedure was repeated for the remaining brands and their respective means and standard deviations were calculated.[50]

Dissolution Rate Test

A 0.1 N HCl was utilized as the dissolution medium and one tablet from each brand of the erythromycin tablet was inserted in the dissolution apparatus set at temperature of 37 ^{o}c. This was carried out at 50 rpm and 5 ml of the various brands was withdrawn at different time intervals.[50] The concentration was determined via UV Spectrophotometer at absorbance of 285 nm along with the blank sample.

Disintegration Rate Test

Six tablets of each brand were placed into the tubes of the disintegration apparatus and suspended in the beaker with distilled water as medium. The time at which the drug samples disintegrated was recorded.[50]

Analysis of Erythromycin tablets

The standard solution containing reference standard was prepared to obtain various concentration of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, and 60 µg/ml using methanol. They were all scanned through UV-Visible wavelength of 200 – 400 nm. A 20 µg/ml concentration of the different brands were prepared and scanned to determine the amounts of erythromycin present in the samples.

Microbiological Assay

The Mueller Hinton agar was prepared according to the manufacturer instructions, and all material required for this procedure was autoclaved at 121^{o}c for 15 mins. The prepared agar was poured into the petri dish and allowed to cool. The microbiological assay of all the brands was determined by evaluating the susceptibility of the various bacterial clinical isolates to the different brands. A 1 ml of the inoculates was withdrawn from the stock with a sterile syringe and transferred into the petri dish containing the Mueller Hinton agar, and even distributed on the surface of the agar plate. A sterile cork-borer was used to bore five holes in the agar plate. The holes were inoculated with 15 mcg/ml of the sample prepared using methanol as solvent. The agar plate containing the various brands were incubated at 37^{o}c for 24 hours and their respective zone of inhibitions was observed and measured.

RESULTS

Table 1: Showing uniformity mean weight, number of tablets deviated and Percentage purity.

Sample	Mean weight (g)	Number of tablets deviated	Percentage Purity (%w/w) ± SD
E1	1.020	Nil	97.4 ± 0. 001
E2	0.831	Nil	98.0 ± 0.004
E3	0.603	Nil	99.7 ± 0.003
E4	0.861	Nil	96. 3 ± 0.004
E5	0.905	Nil	100.5 ± 0.002

**SD = Standard deviation*

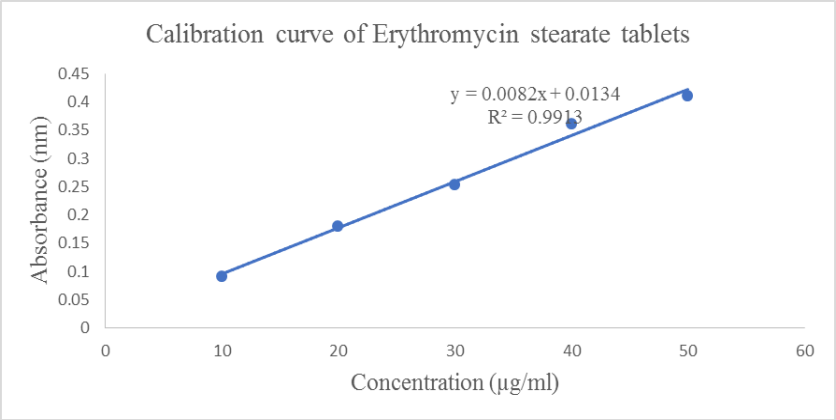

Figure 2: Showing the calibration curve for Erythromycin Stearate tablets.

Table 2: Disintegration Time in Water.

Sample	Mean time (mins) ± SD	CV (%)
E1	5.8 ± 0.4	7.6
E2	20.9 ± 1.5	6.9
E3	11.4 ± 0.7	6.0
E4	13.7 ± 5.1	37.3
E5	8.9 ± 1.0	10.6

*SD = Standard Deviation, CV = Coefficient of Variation

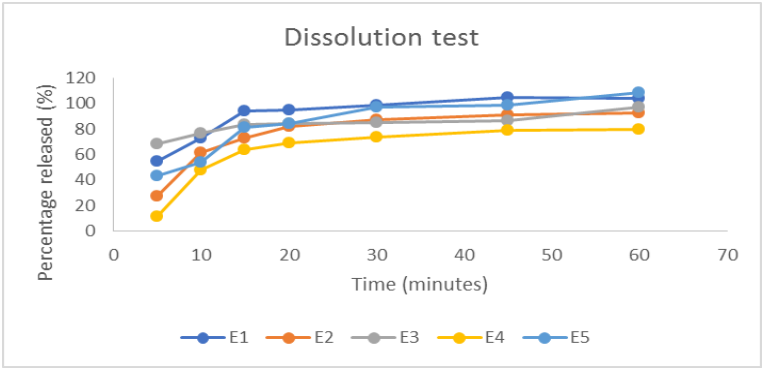

Figure 3: The dissolution test for the various brand of Erythromycin stearate tablets.

Table 3: Zone of inhibition measurement for different brands of erythromycin stearate tablets.

Micro-organisms	Diameter (mm)				
	E1	E2	E3	E4	E5
P. aeruginosa	23	24	26	18	23
E. coli	28	27	21	25	26
B. subtilis	29	28	26	27	27
S. aureus	22	22	20	22	21

DISCUSSION

According to Table 1, the mean weights of the different brands are 1.020 g for E1, 0.831 g for E2, 0.603 g for E3, 0.861 g for E4, and 0.905 g for E5. Since there were no brands with any tablets whose percentage deviation was greater than 5 %, all brands reportedly passed the weight uniformity test.

A stock solution of the reference standard was prepared through serial dilution to obtain various concentrations. These concentrations were then scanned between 200 and 400 nm, with the λ_{max} seen at 285 nm. This data was used to create the calibration curve, which was then used to extrapolate the concentrations of the various brands. The erythromycin UV spectroscopy calibration curve (Figure 2) demonstrates that the medication complies with Beer-Lambert law. With the regression equation y = 0.0082x + 0.0134, the graph is linear. The correlation between the absorbance values and the drug concentration is 99.13 %, as shown by the regressions square R^2 = 0.9913. As it is less than 0.05, the p-value of 0.000348 indicates a strong correlation between absorbance and concentration and excludes chance. The measurements are accurate, as evidenced by the standard error having a low value of 0.014029, and the regression line offers a reliable approximation of the true value. The statistical results for the calibration curve of Erythromycin stearate tablets' UV spectroscopy demonstrate the calibration curve's accuracy, dependability, and reproducibility in estimating the drug concentration in a sample based on its absorbance value. The calibration curve for Erythromycin stearate tablets obtained for UV spectroscopy is therefore suggested to be sufficiently precise, accurate, and reliable in predicting the drug's concentration in samples based on its absorbance value by the values of R^2, p-value, and standard error. The percentage purity and standard deviation of the various brands E1, E2, E3, E4 and E5 are 97.4 ± 0. 001 g, 98.0 ± 0.004 g, 99.7 ± 0.003 g, 96. 3 ± 0.004 g, and 100.5 ± 0.002 g respectively as shown in Table 1. All brands fall within the stipulated range for percentage purity, which is defined by the official compendia as being between 96 and 102 %w/w. All

brands have very low standard deviations, which means that there is little variation between them.

The mean disintegration time (minutes) for the five brands with their standard deviation E1, E2, E3, E4, and E5 are 5.8 ± 0.4, 20.9 ± 1.5, 11.4 ± 0.7, 13.7 ± 5.1 and 8.9 ± 1.0 respectively as shown in Table 3. According to this, E1 is the brand with the shortest mean disintegration time, so it dissolves first. E5 is next, followed by E3, E4, and E2, with the longest disintegration time. E4 has the highest standard deviation value, indicating that there is more variability or inconsistent behavior compared to others, while E1 has the lowest value. The coefficient of variation represents the relative variability of brands to their means with the highest value of 37.3% for E4 which suggests that its disintegration behaviour is more variable when compared to other brands. All samples passed the disintegration test according to the BP Pharmacopoeia because they all disintegrated in less than 30 minutes, between 5 and 20 minutes, on average.

To determine the dissolution profiles of the five brands over a period of 60 minutes, a graph of the percentage of drugs released over time (Figure 3) was plotted. The graph demonstrates how the percentage of drugs released rises over time for all brands, with E3 releasing the most drugs (68.18%) and E4 releasing the least amounts (11.38%) at the five-minute mark. E4 had the lowest drug release at 60 minutes, at 79.91 %, and E5 had the highest drug release at 108.1 %. All brands met the dissolution test standard except for E4 (79.91 % at 60 minutes), which did not dissolve at least 80% of the drugs in accordance with the BP specification.[25]

Antibiotics' antibacterial activity against various microbes is measured by the Zone of Inhibition. According to Table 4, the samples have varying degrees of activity against various microorganisms. The zone of inhibition interpretative standard for erythromycin at a concentration of 15 mcg/ml against microorganisms with a diameter of 13 and below is resistant, intermediates 14 to 22, and 23 and above are susceptible, according to the Kirby-Bauer (antibiotic sensitivity) test. For *P. aeruginosa*, the diameter reveals that all brands, except for E4, have high antibacterial activity, while E4 has an antibacterial activity of 18 which is intermediate antibacterial activity. The brand with the highest antibacterial activity is E3, which has the highest diameter of 26 among all brands. For *E. coli*, the diameter shows that all brands have high antibacterial activity against the organism except for E3 which has diameter of 21, meaning it has intermediate antibacterial activity. E1 has the highest

antibacterial activity with 28, followed by E2 with 27, E5 26 and E4 with 25. For *B. subtilis*, E1 has the highest value of 29 making the organism most susceptible to E1 and the lowest 26 for E3 making it the least effective. As a result, E1 has the highest level of antibacterial activity and E3 has the lowest level of antibacterial activity against the bacteria. The diameter of S. aureus reveals that all brands have intermediate antibacterial activity toward the organism, with E1, E2 and E4 being the most effective with a diameter of 22 and E3 being the least effective with a diameter of 20.

CONCLUSION

All brands of erythromycin stearate tablets passed the weight uniformity test. From the percentage purity, all brands passed the test as their percentage purity was within the stipulated range. All brands disintegrated in less than 30 minutes hence they all passed the disintegration test. Except for E4, which had a dissolution percentage of 79.9 % in the dissolution test, all brands were dissolved to 80 percent in 60 minutes. They were to varying degrees susceptible to all brands for the antimicrobial screening. Against E. coli, all brands had high antibacterial activity except E3 with diameter of 21, which possess intermediate antibacterial activity. E1 had the highest antibacterial activity. All brands had intermediate antibacterial activity against S. aureus, with E1, E2, and E4 being the most effective. Against P. aeruginosa, all brands had high antibacterial activity except E4 with diameter of 18, which possess intermediate antibacterial activity. Against B. subtilis, all brands exhibited high antibacterial activity with the most effective been E1.

ACKNOWLEDGEMENT

Our sincere appreciation goes to the staff of the Department of Pharmaceutical and Medicinal Chemistry and also Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University.

REFERENCE

1. Farzam K, Nessel TA, Quick J. Erythromycin. [cited 2023 Apr]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532249/
2. Wikipedia. Erythromycin [cited 2023 Apr]. In: Wikipedia [Internet]. Available from: https://en.wikipedia.org/wiki/Erythromycin
3. Indian Pharmacoepia. The Indian Pharmacopoeia Commission, Ghaziabad, 2007.

4. Jelić D, Antolović R. (From Erythromycin to Azithromycin and New Potential Ribosome-Binding Antimicrobials). Antibiotics (Basel), 2016; 5(3): 29. doi: 10.3390/antibiotics5030029. PMID: 27598215; PMCID: PMC5039525.
5. The World Bank. Antimicrobial Resistance [cited 2023 Apr]. In: The World Bank [Internet]. Available from: https://www.worldbank.org/en/topic/health/brief/antimicrobial-resistance-amr
6. Tadesse BT, Ashley EA, Ongarello S, Havumaki J, Wijegoonewardena M, González I, Dittrich S. (Antimicrobial resistance in Africa: a systematic review). BMC Infect Dis, 2017; 17: 616. https://doi.org/10.1186/s12879-017-2713-1
7. Samuel K, Kelvin K, Celestine W, Robert O, Cecilia M. (Antimicrobial Resistance Rates and Surveillance in Sub-Saharan Africa: Where Are We Now?). Infection and Drug Resistance, 2022; 15: 3589–3609. https://doi.org/10.2147/IDR.S342753
8. Hayat K, Rosenthal M, Gillani AH, Zhai P, Aziz MM, Ji W, Chang J, Hu H, Fang Y. (Perspective of Pakistani physicians towards hospital antimicrobial stewardship programs: a multisite exploratory qualitative study). Int J Environ Res Public Health, 2019; 16(9): 1565. doi:10.3390/ijerph16091565
9. Institute for Health Metrics and Evaluation (IHME), University of Oxford. Global Bacterial Antimicrobial Resistance Burden Estimates 2019. Seattle, United States of America: Institute for Health Metrics and Evaluation (IHME), 2022. https://doi.org/10.6069/DBG1-V028
10. WHO. Antimicrobial resistance. [cited in 2023 Apr]. In: WHO [Internet] Geneva. Available from: https://www.afro.who.int/health-topics/antimicrobial-resistance
11. Australian Government: Antimicrobial Resistance [cited 2023 Apr]. What causes AMR? In: Australian Government: Antimicrobial Resistance [Internet] Australia. Available from: https://www.amr.gov.au/about-amr/what-causes-amr
12. GBAC: InfectionControl.tips [cited 2023 Apr]. 6 Factors that have caused antibiotic resistance. In: GBAC: InfectionControl.tips [Internet]. Available from: https://infectioncontrol.tips/2015/11/18/6-factors-that-have-caused-antibiotic-resistance/
13. Kelesidis T, Kelesidis I, Rafailidis PI, Falagas ME. (Counterfeit or substandard antimicrobial drugs: a review of the scientific evidence). J Antimicrob Chemother, 2007; 60(2): 214-236
14. Ayush S, Akanksha S, Mukhtar AD, Rimple JK, Jaykaran C, Katia I, Mainul H, Krishna M, Ravichandiran V, Sameer D. (Menace of antimicrobial resistance in LMICs: Current surveillance practices and control measures to tackle hostility). Journal of Infection and

Public Health, 2022; 15(2): 172-181. ISSN 1876-0341,https://doi.org/10.1016/j.jiph.2021.12.008.

15. Ayukekbong JA, Ntemgwa M, Atabe AN. (The threat of antimicrobial resistance in developing countries: causes and control strategies). Antimicrob Resist Infect Control, 2017; 6: 47. doi: 10.1186/s13756-017-0208-x. PMID: 28515903; PMCID: PMC5433038.
16. Lansang MA, Lucas-Aquino R, Tupasi TE, Mina VS, Salazar LS, Juban N, Limjoco TT, Nisperos LE, Kunin CM. (Purchase antibiotics without prescription in Manila, the Philippines. Inappropriate choices and doses). J Clin Epidemiol, 1990; 43(1): 61-67. DOI:https://doi.org/10.1016/0895-4356(90)90057-V
17. Rashidian A, Jahanmehr N, Jabbour S, Shehla Z, Fatemeh S, Maryam B. (Bibliographic review of research publications on access to and use of medicines in low-income and middle-income countries in the Eastern Mediterranean Region: identifying the research gaps). BMJ Open, 2013; 3: e003332. doi: 10.1136/bmjopen-2013-003332
18. Shet A, Sundaresan S, Forsberg BC. (Pharmacy-based dispensing of antimicrobial agents without prescription in India: appropriateness and cost burden in the private sector). Antimicrob Resist Infect Control, 2015; 4: 55. https://doi.org/10.1186/s13756-015-0098-8
19. Okeke IN, Lamikanra A. (Quality and bioavailability of ampicillin capsules dispensed in a Nigerian semi-urban community). Afr J Med Med Sci., 2001; 30(1-2): 47-51. PMID: 14510150.
20. Risha PG, Shewiyo D, Msami A, Masuki G, Vergote G, Vervaet C, Remon JP. (In vitro evaluation of the quality of essential drugs on the Tanzanian market). Trop Med Int Health, 2002; 7(8): 701-707. doi: 10.1046/j.1365-3156.2002.00937.x. PMID: 12167097.
21. Bekele LK, Gebeyehu GG. (Application of Different Analytical Techniques and Microbiological Assays for the Analysis of Macrolide Antibiotics from Pharmaceutical Dosage Forms and Biological Matrices). International Scholarly Research Notices, 2012; 17: 2012. Article ID 859473. https://doi.org/10.5402/2012/859473
22. Sharma R, Agrawal AK. (Development and validation of a high-performance liquid chromatographic method for the determination of erythromycin in tablets). J Pharm Biomed Anal., 2002; 30(3): 611-616.
23. Wardrop J, Ficker D, Franklin S, Gorski RJ. (Determination of erythromycin and related substances in enteric-coated tablet formulations by reversed-phase liquid chromatography). J Pharm Sci., 2000; 89(9): 1097-1105. doi: 10.1002/1520-6017(200009)89:9<1097::aid-jps1>3.0.co;2-d. PMID: 10944374.

24. Mahmoudi A, Boukhechem MS. (Novel Liquid Chromatographic Method for the Simultaneous Evaluation of Erythromycin and Azithromycin in Human Urine). J Materials and Environ Sci., 2017; 8(6): 1953-1959 ISSN: 2028-2508 JMES.
25. Kibwage IO, Roets E, Hoogmartens J. (Thin-layer chromatography of erythromycins and other macrolides). Journal of Chromatography, 1983; 256(1): 164–171.
26. Robertson JH, Tsuji K. (GLC determination of erythromycin in enteric-coated tablets). J Pharm Sci., 1972; 61(10): 1633–1635.
27. Danielson ND. (Simple methods for the qualitative identification and quantitative determination of macrolide antibiotics). J Pharm and Biomed Anal., 1993; 11(7): 121–130.
28. Thanh Ha PT, Van Schepdael A, Roets E, Hoogmartens J. (Investigating the potential of erythromycin and derivatives as chiral selector in capillary electrophoresis). J Pharm Biomed Anal, 2004; 34(5): 861-870. doi: 10.1016/j.jpba.2003.11.004. PMID: 15019020.
29. Bagchi, Anindya. (Development and validation of uv spectrophotometric method for estimation of erythromycin in bulk drug and pharmaceutical formulation). Inter J of recent advances in pharma research., 2015; 5: 71-76.
30. Rattanapoltaveechai R, Vongkom W, Suntornsuk W, Suntornsuk L. (Simple and rapid spectrophotometric method for the analysis of erythromycin in pharmaceutical dosage forms). Journal of Food and Drug Analysis, 2007; 15(1): 2. https://doi.org/10.38212/2224-6614.2437
31. Ali M, Sherazi ST, Mahesar S. (Quantification of erythromycin in pharmaceutical formulation by transmission Fourier transform infrared spectroscopy). Arabian Journal of Chemistry, 2012; 7(6): 1104.
32. Jeremy RE, Ian KH, John W. (Conformational analysis of erythromycin A derivatives: A predictive method using NMR chemical shift and coupling constant data). MRC, 1990; 28(2): 114-118. https://doi.org/10.1002/mrc.1260280205
33. Mordi MN, Pelta MD, Boote V, Morris GA, Barber J. (Acid-Catalyzed Degradation of Clarithromycin and Erythromycin B: A Comparative Study Using NMR Spectroscopy). J Med Chem., 2000; 43(3): 467-474. DOI: 10.1021/jm9904811
34. Veach BT, Mudalige TK, Barnes PJ, Baker CA. (Quantitative Screening Method for Erythromycin and Tylosin in Honey Using RapidFire Mass Spectrometry). Journal of AOAC INTERNATIONAL, 2019; 102(1): 297-301. https://doi.org/10.5740/jaoacint.18-0107

35. Billedeau SM, Heinze TM, Siitonem PH. (Liquid Chromatography Analysis of Erythromycin A in Salmon Tissue by Electrochemical Detection with Confirmation by Electrospray Ionization Mass Spectrometry). J Agri and Food Chem., 2003; 51(6): 1534-1538. DOI: 10.1021/jf0209138
36. Steve B, Daniel RD, Lin L, Eugene B H Jr. (Analysis of erythromycin by liquid chromatography/mass spectrometry using involatile mobile phases with a novel atmospheric pressure ionization source). Rapic communications in mass spectrometry, 2000; 14(3): 156-160. https://doi.org/10.1002/(SICI)1097-0231(20000215)14:3<156::AID-RCM850>3.0.CO;2-K
37. Deubel A, Fandiño AS, Sörgel F, Holzgrabe U. (Determination of erythromycin and related substances in commercial samples using liquid chromatography/ion trap mass spectrometry). J Chromatogr A., 2006; 1136(1): 39-47. doi: 10.1016/j.chroma.2006.09.057. Epub 2006 Oct 2. PMID: 17014855.
38. Veseli A, Mullallari F, Balidemaj F, Berisha L, Švorc L, Arbneshi T. (Electrochemical determination of erythromycin in drinking water resources by surface modified screen-printed carbon electrodes). Microchemical Journal, 2019; 148: 412-418. ISSN 0026-265X, https://doi.org/10.1016/j.microc.2019.04.086.
39. Veseli A, Mullallari F, Arbneshi, T, Balidemaj F. (Electrochemical determination of erythromycin in drinking water resources by screen printed carbon electrodes). IMCS-2018; 730-731. 10.5162/IMCS2018/P2EC.17.
40. Taninaka C, Ohtani H, Hanada E, Kotaki H, Sato H, Iga T. (Determination of erythromycin, clarithromycin, roxithromycin, and azithromycin in plasma by high-performance liquid chromatography with amperometric detection). J chrom. B, Biomed sci and applications, 2000; 738(2): 405-411.
41. Vajdle O, Guzsv ´ any V, Du ´ san ˇ Skori ˇ c,´ Jasmina Anojciˇ c, Pavle Jovanov, Milka Avramov-Ivi ´ c, J ´ anos Csan ´ adi, Zolt ´ an´ Konya, Slobodan Petrovi ´ c, Andrzej Bobrowski, Voltammetric behavior of ´ erythromycin ethylsuccinate at a renewable silver-amalgam film electrode and its determination in urine and in a pharmaceutical preparation, Electrochimica Acta http://dx.doi.org/10.1016/j.electacta.2015.12.207
42. Radičová M, Behúl M, Vojs M, Bodor R, Staňová AV. (Voltammetric determination of erythromycin in water samples using a boron-doped diamond electrode). physica status solidi, 2015; 252(11): 2608-2613. https://doi.org/10.1002/pssb.201552245
43. Huebra M.-J. G. de la, Bordin G, Rodríguez AR. (Comparative study of coulometric and amperometric detection for the determination of macrolides in human urine using high-

performance liquid chromatography). Anal Bioanal Chem., 2003; 375: 1031–1037. https://doi.org/10.1007/s00216-003-1801-9

44. Ganjali MR, Pirzadeh-Naeeni S, Faridbod F, Attar H, Hosseini M, Norouzi P. (Nano-Composite Carbon Paste Electrode and PVC membrane Sensor for Potentiometric Determination of Erythromycin). Int. J. Electrochem. Sci., 2011; 6: 1968-1980
45. LibreTexts Biology:Kirby-Bauer (Antibiotic Sensitivity) [cited 2023 Apr]. In: LibreTexts Biology [Internet]. Available from : https://bio.libretexts.org/Learning_Objects/Laboratory_Experiments/Microbiology_Labs/Microbiology_Labs_I/09%3A_Kirby-Bauer_(Antibiotic_Sensitivity)
46. Lourenço FR, Kaneko TM, Pinto TJ. (Validation of erythromycin microbiological assay using an alternative experimental design). J AOAC Int., 2007; 90(4): 1107-1110. PMID: 17760348.
47. Tserng K, Wagner JG. (Fluorometric determination of erythromycin and erythromycin propionate in whole blood or plasma and correlation of results with microbiological assay). Analy Chem., 1976; 48(2): 348-353. DOI: 10.1021/ac60366a033
48. British Pharmacopoeia Commission. British Pharmacopoeia 2021. London, UK: The Stationery Office, 2020.