



QUALITY ASSESSMENT OF SOME BRANDS OF ERYTHROMYCIN ORAL FORMULATION ON SALE IN GAYA TOWN, GAYA LOCAL GOVERNMENT, KANO STATE, NIGERIA.

*SHEHU, A.A and MUSTAPHA, A.M.

Department of Microbiology, Faculty of Science and Science Education, Kano University of Science and Technology, Wudil

ARTICLE INFO

Keywords:

Quality, Assessment, Erythromycin, oral formulation, sale, Gaya town.

Correspondence author:

e-mail:

*

aashehudbt2012@yahoo.com

Phone:

ABSTRACT

*A study on the quality assessment of some brands of erythromycin oral formulation on sale in Gaya town was conducted. Fourteen (14) samples of erythromycin comprising seven from Nigeria and seven from foreign countries were purchased from pharmacy and chemist shops and then transported to Microbiology laboratory of Kano University of Science and Technology, Wudil for analysis. Chemical identification features and melting point values of the samples were determined by qualitative tests (WHO, 2004) and Internationally Pharmacopoeia (1993) respectively. Antibacterial activity assay by agar disc diffusion method was carried out using susceptibility clinical *Staphylococcus aureus*. Chemical identification test showed that all the samples were confirmed to contain the basic active ingredient of erythromycin and its salt. The melting point values showed that some exceeded the narrow range of melting point from 100-156°C that is ERY₆ had the highest melting point of 180°C while ERY₁₄ had the least melting point of 87°C. The result of the current study indicates concern and this necessitate regular surveillance test be carried out to ensure the circulation of drugs with acceptable quality. Lastly, NAFDAC, researchers, scientists and other related agencies should expedite action in the crusade for effective drug control in Nigeria.*

INTRODUCTION

The microbiological quality of pharmaceutical products is influenced by the environment in which they were manufactured, and by the materials used in their formulation. The micro flora of the final product may represent the contaminants from the raw materials, equipment from which they were made, from the atmosphere, from the person-to-person operating the process or from the final container into which it was packed. Some of the contaminants may be pathogenic whilst some others may grow even in the preservative or antibiotics (Hugo, 2001). Antibiotics which are used in the preservation of pharmaceutical products have been found to be contaminated by

microorganisms because not all antibiotics are active against all microorganisms as such they can survive in both syrup and capsules (BP, 2001). For this reasons, the United States pharmacopoeia, British pharmacopoeia (BP) and the European pharmacopoeia, now use the microbial limit test for evaluation of Good Pharmaceutical Manufacturing Practice (GPMP) rather than a test for routine and product testing (BP, 2001). Regardless of what tests are meant to evaluate, the main problem is that these contaminants are mostly pathogenic, and when consumed, injected or applied to the system may cause serious medicament borne infection macrolides (Baird, 2003).

Group of antibiotics that act against wide range of Gram positive and some Gram negative bacteria are not free from such contamination they are widely used in the treatment of respiratory tract infections and infection caused by some Gram negative anaerobic bacteria such as *Treponemapalladium* (Prescott, 2009). The groups of macrolides available in almost all pharmaceutical shops around the country are erythromycin, lincomycin, clarithromycin, roxithromycin, clindamycin, and azithromycin. Quality of antimicrobial such as erythromycin is an issue in today's health care that concerns everybody (Bernard, 2011). The national agency for foods, drugs administration and control (NAFDAC, 2004) pointed out that the drug distribution in Nigeria has contributed strongly in the sale of poor quality drugs of which antimicrobials are the majority. Several investigators have reported their findings on many antimicrobial such as Metronidazole (Taylor, 2001), Ampicillin (Kolawole, 2002), Chloramphenicol (Wudil, 2004), Cotrimazole (Ohwoavworhua, 2004), and erythromycin (Mukhtar, 2005) all with respect to Nigeria. These studies showed the presence of brand with doubtful quality in circulation. Mukhtar (2005) pointed out that research attention by independent workers, except perhaps NAFDAC and the national institute for pharmaceutical research and development (NIFRAD) with respect to erythromycin in Nigeria seemed to be sluggish despite the importance of the drug in chemotherapy. The world health organization committee for review of medicine has recommended a post market surveillance of all such essential drugs which seemed to be more vulnerable to mutilation or adulteration by fakers (Routledge and Richens, 1990). The aim of the research is to determine the quality assessment and antibacterial activity of some brands of erythromycin oral formulation on sales in pharmacy and patient medicine shops, and the objective is to determine the susceptibilities of some selected erythromycin oral formulation on clinical isolates of *Staphylococcus aureus*.

MATERIALS AND METHODS

The Study Area

Gaya Local Government is the study area and is one of the 44 Local Governments in Kano State. It is approximately between latitude 12°01'N and longitude 8°29'E. Gaya Local Government area was bounded by Jigawa State to the east, by Albasu Local Government to the South, by Wudil Local Government to the west and Ajingi Local Government to the north respectively. It has total area coverage of about 613km². It's headquarter is in Gaya town. According to the national population census 2006, the population of Kano State was estimated to be about 9, 383,682 people and that of Gaya local Government area was 201,016 people. Most of the people are predominantly farmers and also a significant number are involved in businesses of different types including sells of drugs.

Sampling Site

Pharmacy and patent medicine shops located along the main road linking to Maiduguri road of extreme north of Gaya town were surveyed for any available brand of erythromycin oral formulation on sale. The choice of the site was governed by the criterion that, the area used to be main centre for business with many shops dealing with drugs and other pharmaceutical products along the main road. The concentration of these shops was mainly due to the presence of Gaya local government General Hospital in that vicinity that served as the main hospital in the whole of Gaya local Government, Kano State. Thus the shops served as alternative pharmaceutical market for the hospital.

Sampling of the Drugs Used in the Study

Available brands of oral tablets and suspension of erythromycin in the shops were purchased anonymously and immediately transported to Microbiology laboratory of Kano University of Science and Technology, Wudil for analysis, nature of the storage facilities including availability of air conditioning system and ventilation were all noted. Brand name, batch number, NAFDAC registration number, manufacturing and expiry dates, dosage and dose regime, content of formulation, preparation guide and

the name and address of the manufacturers were noted and documented as well as nature of packing was also assessed as required by the British pharmacopoeia (BP, 2001).

Chemical Identification Test for Erythromycin Ethyl succinate

A qualitative test on colour and other reaction specific to erythromycin as described in the WHO (2004) basic tests for pharmaceutical substances were employed. In a set of two test tubes, 60mg of the sample was dissolved in 6ml of distilled deionized water and 3ml of sulphuric acid was added to it. A red brown ring at the interphase which becomes dark red-brown was positive for erythromycin. A lower layer that formed was shaken and the colour produced was noted. To another set of two test tube 9mg of the sample was dissolved in 6ml acetone and 6ml of hydroform was added, partial solubility in acetone, orange coloration on addition of erythromycin ethylsuccinate which later becomes permanent. Another 15mg of the sample was dissolved in 3ml of ethanol and 1.5ml of potassium permanganate was added. A purple solution containing the presence of erythromycin ethylsuccinate.

Test for Erythromycin Stearate

A 100mg of the sample was mixed with 5ml of hydrochloric acid and 10ml of water (giving a yellow colour) was removed by decantation with the help of a separation funnel (Pyrex). The filtrate was then heated with 3ml of 0.1M sodium hydroxide and allowed to cool forming a brown coloration which set to get on cooling. About 10ml of hot water was then added. To 1.0ml of the solution 1.0ml of calcium chloride (May and Baker Nig, Ltd.) was added forming a brown precipitate which is insoluble when HCL was added.

Identification of Erythromycin Based on Melting Point

This physical technique for the identification of erythromycin as described by the international pharmacopoeia 1993. A portion of 50mg of the antibiotic sample was grounded using small pestle and mortar, and placed in dried thin walled electro thermal capillary tubes until a column of 3cm height was formed. The capillary containing the sample was introduced into Gallenkamp melting point apparatus at 5-10°C below the

expected temperature of melting of the substance sought. The temperature at which substance become completely transparent was observed using a mercury in glass temperature (45mm immersion quick-fit GT Britain). The value of the melting point was compared with the standard value specified by WHO (2004) as reported by Donald (1999), thus:

- Melting point of erythromycin ethylsuccinate is 134°C
- Melting point of erythromycin stearate is 100-104°C
- Melting point of erythromycin lactobionate is 150°C
- Melting point of erythromycin estolate is 135-1328°C

Disk agar diffusion method was employed as described by Chessbrough (2000), Scott (1989) and Kamal (1998). Most medical microbiological laboratories used of the disk diffusion technique in which antibiotics incorporated into absorbent disc are applied to the surface of culture plated seeded with the test organism. Antibiotic diffuse rapidly from the disc into the surrounding medium, setting up a concentration gradient of the drug, the edge of a zone of inhibition is formed where the concentration is able to prevent visible growth of the microorganism (Barry, 1991).

Preparation of Antibiotic Disk of 15g Potency

Paper disk of 6.0mm diameter was punched on what man number 1 filter paper. Batches of 100 disk were placed in screw capped McCartney bottles and sterilized at 140°C for 60mm in dry air oven (Gallenkamp, UK). A solution of the drug sample in the dimethylsulphoxide (DMSO) as v/v was made so that a concentration on 1500µg/ml of the drugs was arrived at. A portion of 1ml quantity of the solution was poured on to the 100 batch of sterilized disk and was shaken with shaker (Gallenkamp, UK), for even absorption. At equilibrium of absorption, each disk was quantitatively established to absorb 0.0ml corresponding to 15µg per disk. These are under wet condition in a tight screw capped bottle in a refrigerator before use. Negative control disk containing only DMSO were also prepared. A positive control erythromycin disk was obtained commercially.

Microscopic Identification of *Staphylococcus aureus*

A smear of the isolate was made on a clean and sterile glass slide, the smear was then fixed on a Bunsen flame. Crystal violet (0.5%) was then added which act as a primary stain, followed by addition of lugol's iodine (mordant), counter stain (safranin) was then added after rinsing the mordant with distilled water, then a decolourizer (acetone) was then added and allowed to dry completely. The slide was viewed under violet X100 and X40 oil immersion microscope and the colonies appeared violet and in cocci. Gram positive bacteria retained the violet colour while Gram negative bacteria stained red (Chessbrough, 2000).

Biochemical Identification of *Staphylococcus aureus*

Biochemical test was set to identify organism to species level. Catalase test was used to differentiate between the two Gram positive bacteria that appears in cocci form that is *Staphylococcus* and *Streptococcus*. A smear of the isolate colony was made on a clean glass slide with sterile wire loop. On addition of hydrogen peroxide H₂O₂ bubbles was produced which indicate the presence of *Staphylococcus aureus*.

Standardization of Inoculum for the Assay

Adequate materials from the two separated colonies of the test bacteria was inoculated on to nutrient broth (biotech. UK) and incubated overnight at 35°C. A 1:20 dilution of the overnight broth culture was developed by diluting 0.1 to 20ml with sterile physiological saline (Dana pharmaceutical limited). These matched with a standard turbidity of 0.5 McFarland by comparing with the turbidity of 1% barium sulphate solution (Chessbrough, 2000).

Culture Media

Mueller Hilton agar (E. Merck, Darmstadt) plates and nutrient broth (Biotech, U.K) were used as growth media throughout the assay. These media were prepared according to the manufacturers guide.

Bioassay Procedure

Excess moisture of the Mueller Hilton agar plates were dried for 10-15 in a drier (Gallenkamp UK), the plates were then inoculated with the *Staphylococcus aureus* by carpeting method using sterile swab (Evapon S.S. Nigeria Ltd.).The antibiotic disk were pressed on the surface of the agar plate with sterile pair of forceps. The disks were positioned at least 4mm away from each other and at least 3mm from the edge of the plate. The plates were then inoculated aerobically at 37°C for 18 hours

The sample contained minimal amount of erythromycin when the zone size was within 14-22mm showing intermediate activity the sample was rated as substandard when the zone size is within 8-13mm. absence of erythromycin at all in the sample will be recorded when the zone size is below 8.0mm. This was adopted from the interpretative chart of erythromycin activity of *Staphylococcus aureus* reported by Chessbrough (2000).

Statistical Analysis

The results obtained during the research were subjected to analysis using student T-test (Mukhtar, 2004).

RESULTS AND DISCUSSION

Table 1: Pharmacopoeia Details of Brands of Erythromycin (ERY) Samples in Gaya Town, Gaya Local Government Area

Sample	Batch Number	Expiry Date	NAFDAC Number	Presentation	Manufacturer
Ery1	+1824	06/08/16	A4-3010	125mg Suspension	Nemel Pharmacy Nig. Ltd.
Ery2	017	13/01/17	A4-1050	125mg Suspension	Tuyil Pharm. Nig. Ltd.
Ery3	102	11/03/18	A4-5863	125mg Suspension	Mopson Pharm. Pakistan
Ery4	AZ025	02/05/17	A4-4108	125mg Suspension	Hovid Bhd. Pharm. Malaysia
Ery5	558VA	13/11/19	A4-1704	125mg Suspension	AC Drugs Ltd. England
Ery6	AO4031	02/03/18	A4-4117	250mg Suspension	Strides Vital Pharm.Nig. Ltd.
Ery7	SE-7029	07/07/16	A4-1866	200mg Suspension	Maxheal Lab. Pvt. Ltd. India
Ery8	E-4132	18/01/17	A4-1006	500mg Tablet	Juhel Pharm.Nig. Ltd.
Ery9	470689	30/03/17	A4-3205	500mg Tablet	Labcin Pharm. India
Ery10	0606351	22/04/18	A4-2369	500mg Tablet	New Divine Favor. England
Ery11	6387	29/07/19	A4-8646	500mg Tablet	Rico Pharm. Ind. Nig. Ltd.
Ery12	02007	15/03/16	A4-5476	500mg Tablet	Stearate Ind. Ltd. Nigeria
Ery13	ZZTH0013	15/05/18	A4-2343	500mg Tablet	Strides Vital Pharm. Nig. Ltd.
Ery14	235	14/04/17	A4-0915	500mg Tablet	Maxheal Lab. Pvt. Ltd. India

Key:

Ery = Erythromycin

Table 1 shows the pharmacopoeias details of brands of erythromycin sample around Gaya town. Fourteen (14) brands of erythromycin samples were recorded in the table, out of which seven (7) were in form of suspension and the remaining seven (7) in the form of tablet. The presence of Gaya General Hospital had contributed to the availability of many chemists and pharmacy shops that ensured the sale of drugs. Out of the 14 samples, 7 were foreign and 7 were Nigerian. However, none of the samples was without evidence of NAFDAC registration number which could have made them more vulnerable to suspicion in terms of poor quality and faking or counterfeit characteristics. All the samples had NAFDAC number, manufacturing and expiry date, amount and the country of manufacture.

Storage facilities were mainly not adequate in which only 5 pharmacies had been met with their air conditioning facilities working perfectly. Others used fans at room temperature, which is not in conformity with the 25°C specification (B.P, 2001). From the result recorded, 50% of the samples were from indigene also 50% of them were foreign. This coincides with the findings of Bernard (2011) that much of the essential drugs needed in Nigeria are imported. The nature of packaging however could be used to forecast the quality of erythromycin formulation. For example Ery₁, Ery₂, Ery₅, Ery₈ and Ery₉ were packaged very well and beautifully designed concurrently.

Table 2: Identification of Erythromycin in Samples Based on Colour Reaction and the Melting Point Properties.

Sample	Melting point (°C)	Time of melting point	Presence of Erythromycin	Erythromycin and its base
Ery ₁	177	15	+	Ethylsuccinate
Ery ₂	179	13	+	Ethylsuccinate
Ery ₃	178	12	+	Ethylsuccinate
Ery ₄	179	13	+	Ethylsuccinate
Ery ₅	145	2	+	Ethylsuccinate
Ery ₆	180	8	+	Ethylsuccinate
Ery ₇	178	3	+	Ethylsuccinate
Ery ₈	147	2	+	Stearate
Ery ₉	135	3	+	Stearate
Ery ₁₀	110	2	+	Stearate
Ery ₁₁	115	3	+	Stearate
Ery ₁₂	100	2	+	Stearate
Ery ₁₃	120	2	+	Stearate
Ery ₁₄	87	1	+	Stearate

Key: (+) = colour reaction observed conformed to the true identity of the erythromycin in its labelled vehicle as specified by (WHO, 2004). Ery = Erythromycin.

Table 2 shows the chemical identification test of erythromycin samples based on colour reaction and melting point. Seven (7) brands of erythromycin ethylsuccinate (Ery₁-Ery₇) and the 7 brands of erythromycin stearate (Ery₈-Ery₁₄). Ery₆ had the highest melting point of 180°C followed by (Ery₂-Ery₄) and Ery₃. Even though Ery₁ had melting point less than the above mentioned, but had a greater time for melting point above all the samples which was

up to 15 minutes. The high melting point probably indicated impurities in the samples. Ery₁₄ had the least melting point of 87°C and time of melting point (1 minute), probably because the sample was formulated with some substances that were very volatile. Basic active ingredient of Erythromycin was confirmed in all the 14 samples and this was indicated by (+) sign in the table. Ery₅, Ery₈, Ery₉, Ery₁₀, Ery₁₁,

Ery₁₂, Ery₁₃, and Ery₁₄ confirmed erythromycin stearate at melting point of 87-150°C with the first 1-8 minutes of exposure to the heat with melting point values of 145, 147, 135, 110, 115, 100, 120 and 87°C respectively. The other Ery₁, Ery₂, Ery₃, Ery₄, Ery₆, and Ery₇ with melting points of 177, 179, 178, 179, 180 and 178°C respectively, all melted at a range of time between 12-15 minutes with highest melting point of 180°C. This indicates presence of impurities in the drug samples since the purer the samples the lower the melting point. Lowest melting point could be due to the fact that the drug was formulated together with constituent that could also be volatile. Though chemical assay method is generally more accurate and requires less time than biological

methods, they are still less sensitive and coincides with the work of (Pelczar, 2002). Perhaps that was why in the chemical identity test all the samples appeared to have shown they contained active ingredient of erythromycin but the melting point test showed that only 52% of the samples within the narrow range. This is also in contrast to the current bioassay test which showed that all the samples has acceptable quality. Statistical analysis using rank correlation according to (Pelczar, 2002), showed that there exist a strong positive correlation between the melting point value and the time taken for substance to melt at 0.05 level of significance.

Table 3: Antibacterial Activity of Brands of Erythromycin (ERY) Samples in Gaya Town, Gaya Local Government Area

Drug Sample (15µ disk potency)	Zone of Inhibition (mm)	Activity
Ery ₁	26	++
Ery ₂	25	++
Ery ₃	17	+
Ery ₄	19	+
Ery ₅	29	+++
Ery ₆	25	++
Ery ₇	25	++
Ery ₈	24	++
Ery ₉	27	++
Ery ₁₀	22	++
Ery ₁₁	21	+
Ery ₁₂	17	+
Ery ₁₃	26	++
Ery ₁₄	21	+
Control	31	+++

Key: (+++) = highly active.

(++) = Active

(+) = moderately active

Table 3 shows antibacterial activities of some brands of erythromycin samples around Gaya town. Ery₅ had the highest diameter of zone of inhibition of 29mm and highest activity level of (+++). This could be as a result of high concentration of active erythromycin ingredient. This is followed by Ery₇ with zone

of inhibition of 27mm, Ery₃ and Ery₁₂ had the least diameter of zone of inhibition of 17mm and this indicates the sample contains little active ingredient. Only one of the sample was highly active, 7 are active while the remaining 5 are moderately active. However, none of them was found to be poorly active.

Correlation analysis also at $p < 0.05$ revealed that there is no significant difference between the melting point values and antibacterial activity of the samples. This conforms to the finding of Mukhtar (2005).

T-test as described by Mukhtar (2004) showed that the melting point of erythromycin ethylsuccinate and that of stearate were found to have significant difference at 5% probability, probably because ethylsuccinate are in form of suspension and that of stearate are in form of tablets and their differences in stated amount of mg, whereas bacterial activity using T-test between that of erythromycin ethylsuccinate and that of stearate showed no significant difference. This is because they all contains the active ingredient of erythromycin which means all the samples are susceptible to the targeted organism.

CONCLUSION

The study revealed that erythromycin for both paediatrics and adult formulation was readily available on sale around Gaya town. The samples tested in this study showed the presence of chemical, physical, and antibacterial qualities and activity with varying degrees of moderate active and few high active of different brands of oral formulation of erythromycin dispensed in Gaya town during the period of this study.

The test organism *Staphylococcus aureus* also showed susceptibility to all the samples

tested. It can therefore be concluded that erythromycin oral formulation in circulation around the study area included largely of acceptable quality ones. The test organism *Staphylococcus aureus* also showed susceptibility to all the samples tested.

RECOMMENDATIONS

Based on the study, the following recommendations were made:

1. Regular surveillance test should be carried out to ensure the circulation of drugs with acceptable quality
2. Local pharmaceutical companies should be more careful on the need to take precautions to avoid contamination during processing.
3. National Agency for Food and Drug Administration and Control (NAFDAC), researchers, Scientists and other related agencies should expedite action in the crusade for affecting drug control in Nigeria.
4. Workshops on the need to improve the quality of erythromycin should be organized.
5. Funding of such research and related workshops should be sponsored by pharmaceutical industries to ensure the circulation of standard drugs.
6. Good ethical relationship should be maintained between pharmaceutical companies, prescribers and pharmacists.

REFERENCES

- British Pharmacopoeia (BP, 2001). Standard method for Assay for quality of antimicrobials, Her majesty stationary office, London. **11**:774-903.
- Baird, R.M (2003). Microbial contamination of pharmaceutical products made in a hospital Pharmacy Clinic. **10**: 95-100.
- Bernard, P; Peirre, T and Jackques, P (2011). Access to essential drugs in poor countries. A list battle. *The J of the American medical Association*. **281**:361-367. www.jama.com.
- Barry, S (1991). Disk diffusion method. *Microbiology and microbial infection*. 223pp.
- Chessbrough, M (2000). *Medical Laboratory Manual for Countries*. Vol 313. Churchill Livingston, UK.
- Donald, L.P (1999). *Introduction to organic laboratory ethics* 3rd edition. Western Washington University, USA. Pp 535-527.
- Hugo, W.B and Russell, A.D (2001). *Pharmaceutical Microbiology*. Blackwell Scientific Publication, 6th Edn. Pp 339-377.

- Kolawole, J.A (2002). Quality and availability of Ampicillin products in Jos, Nigeria. *Journal of pharm.* **33**:27-42.
- Kamal, M.L (1998). Medical laboratory technology. Vol. 2 McGraw Hill publishing Company, New Delhi India. Pp612-755.
- Mukhtar, M.D (2005). Invitro Assessment of the chemical and anti-bacterial quality of oral Formulation of erythromycin dispensed in Kano South. *Journal of Medical and pharmaceutical Science.* **2(2)**:10-15.
- Muktar, B.F (2004). Introduction to Biostatistics. Pp 45-51. By Samaris publishers. National Food and Drug Administration and Control (NAFDAC, 2004). Assessment of poor quality drugs. **10**:1-15.
- Ohwoavworhua, F.O (2004). Comparative Invitro Studies of some commercial products of Cotrimaxazole tablets available in Nigeria. Proceeding of the West African Society for pharmacology, 31st Annual Regional Conference, Department of Pharmacology. Faculty of Medicine, Bayero University, Kano.11-16th Oct. 2004. 19pp.
- Prescott, K; Harley, O and Klein, B (2009). Text book of microbiology, 5th edition. McGraw-Hill publishers, USA. Therapeutic Drug Monitoring. 11:47-52.
- Pelczar, M.J (2002). A textbook of microbiology. International edition McGraw Hill Inc. New York. Pp 806-833.
- Routledge, P.A and Richen, A. (1990). Clinical pharmacology. Licensing Authority and Drug control. In: Sauhamy, R.L and Moxharm, J. (1990). Eds textbook of medicine 1st edition, Longman press limited. Pp29-30.
- Scott, A.C (1989). Laboratory control of antibacterial therapy in: Markie and Mac Cartney (1989). Practical medical microbiology. 13th edition, Churchill Livingston, New York. Pp161-180.
- Taylor, R.B (2001). Pharmacopoeias quality of drugs supplied by Nigerian pharmacies lancet 375:1936 in Africa Health **24(1)**:17
- Wudil, A.M (2004). Quality Assessment of the Incidence of Substandard Chloramphenicol Capsule and Injection sold in Kano South. *Biological & Environmental Sci. of Tropic Journal (BEST Journal).* **1(2)**:78-80.
- World Health Organization (WHO, 2004). Basic test for pharmaceutical substances. Macmillan, England. Pp72-74.