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Article

Quality Assessment of Different Brands of Ceftriaxone Injection in Mbabane Retail Outlets

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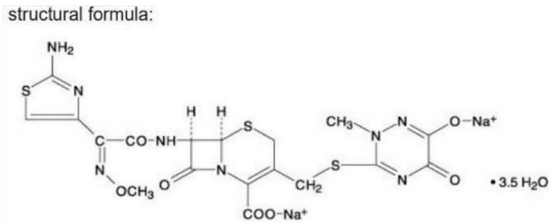
Abstract: Antimicrobial drug resistance (AMR) is a growing global concern associated with the overuse and abuse of antimicrobial agents in clinical settings. Many developing countries like Eswatini still lack an adequate mechanism of checking the quality of purchased pharmaceuticals which perpetrates the spread of substandard or counterfeit drug products. The aim of this study is to evaluate the quality of three different brands of ceftriaxone in Mbabane retail outlets. With these drugs high rate of clinical use, there is a need to evaluate its compliance with stipulated standards in pharmacopoeia for quality assurance. The three different brands of Ceftriaxone sodium were tested with both physical tests (pH, expiry date, label, colour, odour, packaging material) and biological test (susceptibility test) to determine their quality. The three brands passed both the physical and biological tests. None of the drugs were found to be out of the stipulated guidelines of the different Pharmacopoeias. The brands are within the acceptable quality. Based on the results obtained, the brands tested meet the quality requirements based on the quality standards in the Pharmacopoeias.

Keywords: Antimicrobial drug resistance, antimicrobial agents, susceptibility, quality assurance, compliance, pharmacopoeias

1. Introduction

Quality control and assurance of medicinal products is a very vital necessity of pharmaceutical products [1,2]. With the recent high rise of Ceftriaxone antibiotic use in Eswatini, retail pharmacies in the country have fallen under the expectation of FDA standards for food regulation as there is no government established office for Good Manufacturing practice (GMP), or quality control and assurance of drugs being imported into the country [3]. Ceftriaxone Sodium is a 3rd generation Cephalosporin antibiotic with the chemical formula of C18H16N8Na2O7S3•3.5H2O and chemical name (6R, 7R)-7-[2-(2-Amino-4thiazolyl)glyoxylamido]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-as-triazin-3-yl)- thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-car-boxylicacid,72-(Z)-(Omethyloxime), disodium salt, ses-quaterhydrate[4,5]. It has a calculated molecular weight of 661.60 and the following structural formula [7].



Ceftriaxone Sodium has a broad spectrum of antimicrobial action that includes the majority of the clinically significant microorganisms: Gram-positive, Gram-negative, aerobic, anaerobic, and blue-pus bacillus [8]. It is resistant with respect to most beta lactamases of Gram-positive and Gram-negative bacteria. It is used for peritonitis, sepsis, meningitis, cholangitis, emphysema of the gall bladder, pneumonia, lung abscesses, pyelonephritis, infections of the bones, joints, skin, soft tissues, abdominal and gynaecological infections, and for infected wounds and burns. The main synonym of this drug is rocefin [6,9]. Ceftriaxone is characterized by in-vitro action against both gram positive and gram negative aerobic and anaerobic bacteria. The bactericidal activity of Ceftriaxone results from inhibition of cell wall synthesis. Cephalosporins exert bactericidal activity by interfering with bacterial cell wall synthesis and inhibiting cross-linking of the peptidoglycan. The

cephalosporins are also thought to play a role in the activation of bacterial cell autolysins which may contribute to bacterial cell lysis. Saturable plasma protein binding within the therapeutic range (the free fraction of ceftriaxone remaining relatively constant at approximately 5 to 10 percent at ceftriaxone plasma concentrations of less than 200 mcg/mL, and increasing to approximately 40 percent at 650 mcg/mL) [10]. No active secretion by renal tubules, and approximately 55 percent renal elimination and 45 percent excretion through the biliary pathway. Ceftriaxone is 95% protein bound with its elimination half-life at 8.8 hours and penetrate into the CSF in sufficient concentration.

Following intramuscular injection, mean peak plasma ceftriaxone levels are approximately half those observed after intravenous administration of an equivalent dose [11]. The maximum plasma concentration after a single dose of 1g is about 81 mg/l and is reached in 2-3 hours after administration. After intravenous administration of ceftriaxone 1g, the mean peak plasma levels is 200mg/l. after intravenous infusion of ceftriaxone 1g, plasma levels are approximately 150 mg/l. The volume of distribution of ceftriaxone is 712 L. concentrations well above minimal inhibitory concentrations of most relevant pathogens detected in tissue including lung, heart, and tonsil, prostatic and synovial fluid. 8-15% increase in peak plasma concentration is seen on repeated administration and steady state is reached in most cases within 48-72 hours depending on the route administration. Ceftriaxone penetrates the meninges [11]. Penetration is greatest when the meninges are inflamed. Mean peak ceftriaxone concentrations in CSF in patients with bacterial meningitis are reported to be up to 25% plasma levels compared to 2% of plasma levels in patients with uninflamed meninges. Ceftriaxone crosses the placental barrier and is excreted in the breast milk at low concentration [11].

Ceftriaxone is used in the treatment of the following infections when caused by susceptible strains of the designated microorganisms: Lower Respiratory Tract Infections caused by E. coli, H. influenzæ, K. pneumoniae and species, Staph. aureus, Strep. pneumoniae and species (excluding enterococci) [6]. Urinary Tract Infections (complicated and uncomplicated) caused by E. coli, Klebsiella species, P. mirabilis and P. vulgaris. Bacterial Septicemia caused by E. coli, H. influenzæ, K. pneumoniae, Staph. aureus and Strep. pneumoniae, (excluding enterococci). Skin and Skin Structure Infections caused by K. pneumoniae and species, P. mirabilis, Staph. aureus, Staph. epidermidis and Streptococcus species (excluding enterococci). Bone and Joint Infections caused by Staph. aureus, Strep. pneumonia and Streptococcus species (excluding enterococci) [6]. Intra-Abdominal Infections caused by E. coli and K. pneumoniae. Meningitis caused by H. influenzæ, N. meningitidis, and Strep. pneumoniae. Ceftriaxone Injection, USP should not be used for the treatment of meningitis caused by L. monocytogenes. Ceftriaxone is the drug of choice for all forms of gonorrhoea and for severe forms of Lyme disease [6].

2. Materials and Methods

2.1. Materials

Three different generic brands of ceftriaxone sodium ampoules were collected from different approved Retail Pharmacies in Mbabane. The different brands were Mvsef, Scotxone and Generics Plus, each brand with a strength of 1g. Nutrient Agar, distilled water, sterile saline in 2-ml tubes, 0.5 McFarland standard, Whatman Filter paper 2, Mueller-Hinton agar plates 100 mm, sterile Swabs, sterile saline and bacterial Isolates – Staphylococcus aureus were purchased from local company Sigma Aldrich.

2.2. Testing Methods

To confirm the safety, quality and efficacy and effectiveness of the three brands of Ceftriaxone sodium ampoule, (each was labelled according to brand name), and quality evaluation test was performed and carried out in this study. Quality evaluation test for Ceftriaxone injection includes physical tests, efficacy tests (susceptibility). The tests for quality of Ceftriaxone sodium ampoule, USP and Pharmacopoeia were used as standard for the evaluation of this study.

2.3. General appearance of ampoule (Physical test)

Testing of general appearance involves measurement of attributes such as; Production date and expiry date, packaging material, shape and dimensions of ampoule, colour and Odor, quality of sealing, pH values to be determined by pH meter.

2.4. Microbiological Assay

Antibiotic susceptibility assays were conducted on the three brands of ceftriaxone using cultured medium, Muller Hinton Agar plates, containing direct colony suspension that was equivalent to 0.5 McFarland Standard for the bacterial isolate Staphylococcus aureus, injected with ceftriaxone and incubation at 35 ºC for 16-18 hours. The diameter of the Zones of growth inhibition produced by the different brands were measured [12, 13].

2.5. Negative Testing (Control)

For Quality control S. aureus ATCC 25923 is used as negative control with culture media and water where it shows no development of zone of inhibition. If there was microbial growth on the control test, it would mean that the sample was not sterilized properly.

3. Results

3.1. Physical Test Results

Table 1. Result of the physical tests conducted on the different samples.

Test	Product Sample		
	Generics Plus	Mvsef	Scotxone
Date of Manufacture	03/23	04/23	06/23
Expiry Date	05/26	05/26	03/26
Colour	Yellow Pale Powder with	Yellow Pale Powder with	Yellow Pale Powder with
	Clear Sterilized water	Clear Sterilized water	Clear Sterilized water
	Light yellow to clear colour mixture after mixing	Light yellow to clear colour mixture after mixing	Light yellow to clear colour mixture after mixing
Odour	Mild	Mild	Mild
pH	6.88	6.72	6.84
Quality of Sealing	Plastic –Water container and Transparent Bottle with no visible impurities	Plastic –Water container and Transparent Bottle with no visible impurities	Plastic –Water container and Transparent Bottle with no visible impurities
	No evidence of being tampered with	No evidence of being tampered with	No evidence of being tampered with

3.2. Biological Test Results

Table 2. Result of the biological tests conducted on the different samples

Day 1			
	Generics Plus	Mvsef	Scotxone
Readings	25 mm	24 mm	24 mm
Day 2			
	Generics Plus		
Readings	23 mm	22 mm	23 mm
Day 3			
	Generics Plus	Mvsef	Scotxone
Readings	23 mm	30 mm	27 mm

Table 3. Show the antimicrobial susceptibility testing – zone size interpretative chart, based on results obtained using Mueller Hinton agar [14].

Zone Size Interpretative Chart (as per CLSI & EUCAST)

Antimicrobial Susceptibility Testing - Zone Size Interpretative Chart

Based on Results obtained using Mueller Hinton Agar

Product Code	Antimicrobial Agent	Symbol	Disc content	Interpretative Criteria			Quality Control Limits (mm)											
				Sensitive mm or more	Intermediate mm	Resistant mm or less	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 35218	*S. aureus* ATCC 29213	*E. faecalis* ATCC 29212	*H. influenzae* ATCC 49247	*H. influenzae* ATCC 49766	*K. pneumoniae* ATCC 700603	*N. gonorrhoeae* ATCC 49226	*S. pneumoniae* ATCC 49619	*C. jejuni* ATCC 33560
SD209	**Cefprozil**	CPR	30 mcg															
	Enterobacteriaceae,																	
	Haemophilus influenzae &																	
	Haemophilus parainfluenzae			18	15–17	14	21–27	27–33	-	-	-	-	-	20–27	-	-	25–32	-
SD062	**Ceftazidime**	CAZ	30 mcg															
	Enterobacteriaceae, B. cepacia			21	18–20	17	25–32	-	-	-	-	-	-	-	10–18	-	-	-
	Paeruginosa, Acientobacter & *Staphylococcus spp.*			18	15–17	14	-	16–20	22–29	-	-	-	-	-	-	-	-	-
	Haemophilus influenzae & *Haemophilus parainfluenzae*			26	-	-	-	-	-	-	-	-	27–35	-	-	-	-	-
	Neisseria gonorrhoeae			31	-	-	-	-	-	-	-	-	-	-	-	35–43	-	-
SD062A	**Ceftazidime**	CAZ	10 mcg															
	Enterobacteriaceae			22	19–21	19	23–29	-	-	-	-	-	-	-	6–12	-	-	-
	Pseudomonas spp.			17	-	17	-	-	21–27	-	-	-	-	-	-	-	-	-
SD110	**Ceftizoxime**	CZX	30 mcg															
	Enterobacteriaceae			25	22–24	21	30–36	27–35	12–17	-	-	-	-	-	-	-	28–34	-
	Haemophilus influenzae & *Haemophilus parainfluenzae*			26	-	-	-	-	-	-	-	-	29–39	-	-	-	-	-
	Neisseria gonorrhoeae			38	-	-	-	-	-	-	-	-	-	-	-	42–51	-	-
SD065	**Ceftriaxone**	CTR	30 mcg															
	Enterobacteriaceae			23	20–22	19	29–35	-	-	-	-	-	-	-	16–24	-	-	-
	Paeruginosa, Acientobacter & *Staphylococcus spp.*			21	14–20	13	-	22–28	17–23	-	-	-	-	-	-	-	-	-
	Haemophilus influenzae & *Haemophilus parainfluenzae*			26	-	-	-	-	-	-	-	-	31–39	-	-	-	-	-
	Neisseria meningitidis			34	-	-	-											
	Neisseria gonorrhoeae			35	-	-	-	-	-	-	-	-	-	-	-	39–51	-	-
	Streptococcus spp. Viridans group			27	25–26	24	-	-	-	-	-	-	-	-	-	-	30–35	-
	Streptococcus spp. *beta haemolytic group*			24	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Enterobacteriaceae			25	22–24	22	29–35	-	-	-	-	-	-	-	16–22	-	-	-
	Streptococcus spp viridans group			27	-	27	-	-	-	-	-	-	-	-	-	-	32–38	-
	Haemophilus influenzae			31	-	31	-	-	-	-	-	-	-	34–42	-	-	-	-
	Moraxella catarrhalis			24	21–23	21	-	-	-	-	-	-	-	-	-	-	-	-
	Kingella kingae			30	-	30	-	-	-	-	-	-	-	-	-	-	-	-

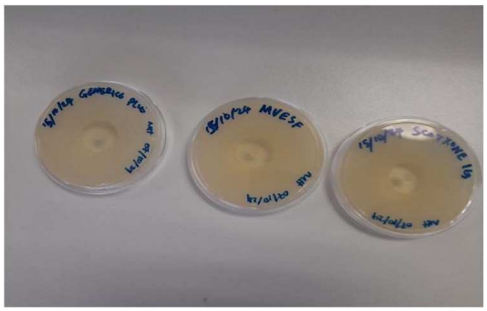


Fig. 1. Day 1 Susceptibility results, showing the zones of inhibition on day one.

Fig. 2. Day 1 Susceptibility results, showing the zones of inhibition on day one.

Fig. 3. Day 3 Susceptibility results, showing the zones of inhibition on day three.

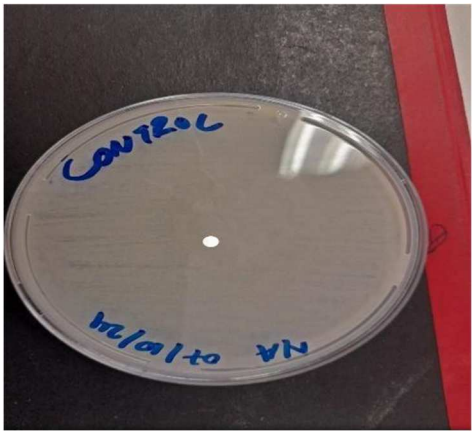


Fig. 4. Showing the control with no zone of inhibition after day three.

4. Discussion

It is expected that every country that has pharmaceutical products should have quality control and assurance department to ensure the precise production of pharmaceutical medicines. Unfortunately, Eswatini currently lacks the incentives of both quality control and assurance. This puts the nation to a high risk of influx of counterfeit and sub-standard drugs which has no guarantee the safety, purity, efficacy, and quality. This study

used information obtained from pharmacopeia to perform quality evaluation of three different brands of Ceftriaxone Sodium ampoule 1gm obtained from local retail pharmacy in Mbabane, Eswatini. The three different brands of Ceftriaxone sodium ampoules were obtained or collected, each with a strength of 1gm. The ampoules were randomly purchased from different selected retail pharmacy outlets in Mbabane and they were within their expiry date. The brand names were used to differentiate them. Data for physical testing and biological testing were collected from the experiments performed and results were presented in tabular format.

4.1. Physical test

Data for physical testing were collected from the experiments performed and results were presented in tabular format as shown in table 3.1. The Pharmacopeial standards, such as those set by the United States Pharmacopoeia and European Pharmacopoeia stipulates that in seal integrity, upon visual inspection: Ampoules should be inspected visually for any sign of cracks, chips, or defects in glass that could compromise the seal. Drug container was transparent bottle with no visible impurities. The Packaging material was acceptable according to the USP as the quality of sealing of all three ceftriaxone ampoules were intact and without any evidence of tampering which could alter the contents. The colour of the Ceftriaxone powder before mixing was yellow pale powder which also had clear sterile water for the three ceftriaxone was acceptable according to the Indian and US pharmacopoeia. Also after mixing the ceftriaxone powder with the sterile water, a light yellow solution was observed which signifies that it passes the USP stipulation. Additionally, both the Indian and US Pharmacopoeia stipulates that Ceftriaxone solution should have a pH range 6.07.0. The study showed the different brands pH as follows; Generics Plus (pH 6.88), Mvsef (pH 6.72) and Scotxone (pH 6.84). This shows that the brands were within the stipulated guidelines, therefore pH was as approved.

4.2. Biological test

Table 2 shows biological test results. To obtain the results for biological testing, the microbes were grown in a suitable media (with nutrient agar) and properly sterilized using an autoclave as per requirements of USP. The medium adhered to the stipulated guidelines. The results in Table 2 are susceptibility test results after measuring the Zone of Inhibition. The table shows that the zones of inhibition for the three brand samples were as follows;

Table 4. Outlines the interpretation chart of the results.

Generics Plus Zone of inhibition
Day 1 (25 mm), Day 2 (23 mm) and Day 3 (23) showing an average of 23.7 mm
Mvsef Zone of inhibition
Day 1 (24 mm), Day 2 (22 mm), Day 3 (30 mm), showing an average of 25.3 mm
Scotxone Zone of inhibition
Day 1 (24 mm), Day 2 (23 mm), Day 3 (27 mm) showing an average of 24.7 mm

It stipulates that for Ceftriaxone susceptibility should be 21 mm or more, Intermediate 14 mm- 20 mm and Resistant should be 13 mm or less [14]. Results for quality control limits for Staphylococcus aureus ACC 25923 strain should be between 22 mm -28mm. The results obtained from this study shows that Generics Plus which had an average of 23.7 mm, was susceptible. Mvsef which had an average zone of inhibition of 25.3 mm was also susceptible. Scotxone which had an average zone of inhibition of 24.7 mm was highly susceptible as well. This susceptibility results implies that Generics Plus, Mvsef and Scotxone were approved as per CLSI and EUCAST standards, therefore passes the test of efficacy and are within the quality control limits [15]. Result of the control medium is shown by Figure 4 in which there was no Zone of Inhibition around the disk impregnated with sterilized water. This means that the medium was sterile, it had no contaminants in it. The analysis on physical tests and biological test on ceftriaxone samples met the established pharmacopeial standards, indicating that they are suitable for clinical use.

5. Conclusion

Quality assurance guarantees drug safety and effectiveness. The quality assessment of Ceftriaxone showed significant insights into its pharmaceutical properties including efficacy, purity and stability.

Ceftriaxone antibiotic is listed amongst the classes of drugs listed as essential medicines in Eswatini [16]. According to the finding of this study of quality assessment of different brands of Ceftriaxone sodium it can be concluded that three brands of ceftriaxone products are equivalent and are of good quality. The findings of this study highlights the importance of quality control measures in the production and distribution of pharmaceutical products. Inconsistent antibiotic quality can lead to treatment failure, multidrug resistance and adverse patient outcomes. Therefore, ensuring that all ceftriaxone products meet stringent quality standards is critical for safeguarding public health.

Conflicts of Interest: The author has no conflict of interest related to this study to disclosure.

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