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Research Article

Low Antibacterial Potency of Ceftriaxone Brands in Mbarara Municipality, Uganda

Anthony Ssebagereka*

School of Public Health, College of Health Sciences, Makerere University, Uganda

***Corresponding Author:** Anthony Ssebagereka, School of Public Health, College of Health Sciences, Makerere University, Uganda.

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Abstract

Introduction: Antibiotic therapy has for a long time been a critical aspect of health care. Ceftriaxone is widely used for empirical antibacterial therapy among Healthcare facilities in Uganda. Antibacterial potency of ceftriaxone is the relative measure that compares doses of different brands required to produce the same bactericidal effect. Reports of widely differing antibiotic activity and clinical outcomes for ceftriaxone brands, from medical professionals, has raised many questions about their potency. The study aim was to determine the relative potency of the different ceftriaxone brands compared to the innovator brand.

Methods: Eleven Ceftriaxone brands on the market were obtained from the pharmacies and drug shops in Mbarara Municipality, including two brands obtained from the public hospital supply chain. Broth macro-dilution technique were used to determine the MIC (Minimum inhibitory concentration) and consequently, the MBC (Minimum Bactericidal Concentration) for each ceftriaxone brand against *Escherichia coli* (representative of gram negative bacteria) and *Staphylococcus aureus* (a representative of gram positive bacteria). Thereafter, the potency ratio (MBC of Innovator brand: MBC of a given ceftriaxone brand) was calculated.

Results: Generally, all the brands were found to be unreasonably less potent than the innovator (reference) brand. However, two brands had the lowest potency ratios (0.25% and 0.5%, respectively against *Escherichia coli* and 5% against *Staphylococcus aureus*), while the innovator brand and one other brand were the most potent brands (50% against *Staphylococcus aureus* and 100% against *Escherichia coli*), exhibiting the lowest MBCs. The two brands supplied in the public hospital, had percentage potency ratios of 10% and 40%, respectively against *Staphylococcus aureus* and 10% and 2.5%, respectively against *Escherichia coli*.

Conclusion: The ceftriaxone brands exhibited widely varying antibacterial activity with inferior bactericidal properties compared to the reference brand. Most of the ceftriaxone brands were largely bacteriostatic rather than bactericidal. In addition, the generic brands generally exhibited much lower potency against *Staphylococcus aureus* than *Escherichia coli* when compared with the reference brand. Further investigations ought to focus on quantification of the Active ingredient, chemical purity, and in-vivo activity of ceftriaxone brands, as well as monitor and assess efficacies and potencies for medicines especially antibiotics.

Keywords: Ceftriaxone; Anti-Bacterial Agents; Microbial Sensitivity Tests; Treatment Outcome

Abbreviations

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; AST: Antimicrobial Susceptibility Tests; ATCC: American Test Culture Centre; MRRH: Mbarara Regional Referral Hospital; WHO: World Health Organization; EC: Effective Concentration; ED: Effective Dose

Background

Ceftriaxone is a broad spectrum, β-lactam antibiotic of third generation Cephalosporins [1]. Ceftriaxone is the single largest cephalosporin with a global market of US $1.3 billion out of the total cephalosporin market of US$9.85 billion [2]. This remarkable

cost has resulted in a massive production and use of various brands of ceftriaxone from different manufacturers, with the aim of trying to ensure unlimited access to cheaper treatment options.

Bacteriostatic activity refers to the inhibition or retardation of bacterial multiplication, while bactericidal activity refers to death of bacteria [3]. MIC is the lowest concentration of antimicrobial agent that inhibits visible growth of micro-organisms after overnight incubation and also gives a quantitative estimate of microbial susceptibility [4]. The MBC is recognized as the standard quantitative index of bactericidal potency [5]. MBC refers to the lowest concentration of an antimicrobial agent that will prevent growth of an organism after sub-culture on to antibiotic-free media" [6].

Potency is a measure of a drug's activity in a biological system that compares different doses of different drugs needed to produce the same effect and is defined as the dose of drug required to produce a specific effect of given intensity as compared to a standard reference [7,8]. The potency of a drug, as a measure of a drug's activity in a biological system, can be a vital factor in determining which drug best serves a patient's needs [9]. Potency is used to compare drugs with similar effects. "Relative potency" refers to a comparison of the required quantities of two drugs; an already standard (reference) drug and a newer test drug, to produce the same defined effect [10]. Selection of appropriate antimicrobial therapy requires a thorough understanding of the likely microbial cause of the infection, local susceptibility patterns, as well as the properties of the antimicrobials available for treating these infections, especially potency, among others [11].

Pharmacologic potency can largely determine the administered dose of a given drug product [12], thus enabling medical practitioners to administer the appropriate doses of the drug to the patient. This helps to streamline therapy, and ensure better health outcomes for the patients. The road Back Foundation further gave a warning that it should not to be assumed that all drugs with the same 'traditional' title are equal and will have the same clinical effect, even though many drug representatives say they are equal [13,14]. Thus, it is necessary to consider the potency of a drug when choosing treatment options for better patient outcomes.

It is expected that generic versions of the same drug should have the same properties, including their potency; that is, equipotent(equally capable of producing a pharmacologic effect of a specified intensity) [15]. Pharmaceutical manufacturers have revealed that some products lose their potency over time, which may account for some variability, in treatment outcomes, with the use of certain products [16]. The AST results help predict the clinical outcome or response to therapy when the antibiotic is administered [17].

Undocumented reports among medical practitioners as well as patients in Mbarara Regional Referral Hospital (MRRH) claim that the administered Ceftriaxone doses, as recommended, are no longer effective. Some practitioners attributed this to suggested lower potency of the ceftriaxone brands on the market, while others have attributed it to possible development of resistance by microbes to the drug. However, Scarborough and colleagues reported that ceftriaxone that there are so far limited reports of resistance to the drug [18].

Considering the critical importance of anti-infective chemotherapy in terms of disease control and emergence of resistance, it is necessary to test the assumption that pharmaceutical equivalent generics are also therapeutic equivalent to innovators [19]. Increasing complaints of dose related therapeutic inefficiencies of ceftriaxone by medical practitioners have raised concerns about the potency of some the brands sold on the market. Therefore, the study was aimed at determining the potency of the different brands of ceftriaxone on the market in Mbarara municipality, Uganda and making a comparison between their respective potency ratios.

Methods

This was a cross sectional study where 20 pharmacies were randomly sampled to obtain the brands of Ceftriaxone on the market, followed by laboratory analysis at MUST Microbiology laboratory. The study was done in Mbarara municipality, located in Mbarara District in South-western Uganda. Mbarara University of Science and Technology (MUST) Microbiology Laboratory, a state-of-the-art microbiology laboratory, and Mbarara Regional Referral Hospital (MRRH) are also located in Mbarara Municipality.

Broth macro-dilution method was used because it allows better quantification of the concentrations of the reconstituted drug that is subjected to the bacteria. Therefore, Standard microorganisms (of known MIC and MBC values) were subjected to different brands

of ceftriaxone obtained from the market, including the brand from the Hospital (supplied by the National Medical Stores to all Public health facilities). Two standard bacteria were used; *Escherichia coli* ATCC 25922 (to represent the gram negative bacteria) and *Staphylococcus aureus* ATCC 25923 (to represent the gram positive bacteria). The standard bacteria used were obtained from MUST Microbiology laboratory.

Serial broth dilutions

For each brand of ceftriaxone (1g) under test, reconstitution of the sterile powder was done by adding 10mls of sterile Normal saline to the sterile powder in the vial (of each brand of the drug) using calibrated micropipettes to yield 100 mg of the drug per ml and the resultant clear solution was diluted, one thousand times (by transferring 1 ml of drug into a sterile bijou bottle containing 9 mls of broth medium and from this resultant homogenous solution, 1 ml was drawn and added to another sterile bijou bottle containing 9 mls of broth medium; this procedure was repeated for the third sterile bijou bottle also containing 9 mls of broth medium), then doubling dilutions were thereafter done in the rest of the ten bijou bottles (by removing 1ml of resultant solution and add it to the sterile bijou bottle containing 1ml of broth medium and mixed thoroughly well, and this procedure was repeated for the rest of the nine sterile bijou bottles). In the last (tenth) bijou bottle, 1 ml of solution was removed and discarded. During serial dilutions, thorough mixing of the solution was ensured.

To each of the of the ten sterile bijou bottles containing 1 ml of broth medium, 1 ml of ceftriaxone and 200µl of 0.5McFarland standard of bacteria were added respectively. The resultant mixture was incubated, at the temperature of 37°C for 24 to 48 hours. The MIC for each brand against the respective standard bacteria under assay, given by the concentration in the tube with the lowest dilution of the ceftriaxone that did not yield turbidity, was noted. A summary of the three sets of serial dilutions used is illustrated in figures 1, 2, and 3.

The dilution adjustments were made gradually for the cases where there were no MIC and MBC values detected. This was so due to the poor antibacterial properties of most of the brands assayed, as it was noted that they only inhibited bacterial growth but did not actually kill the bacteria at the respective subjected concentrations or dilutions. This was evidenced by the high MBC values that these brands had against the respective bacteria.

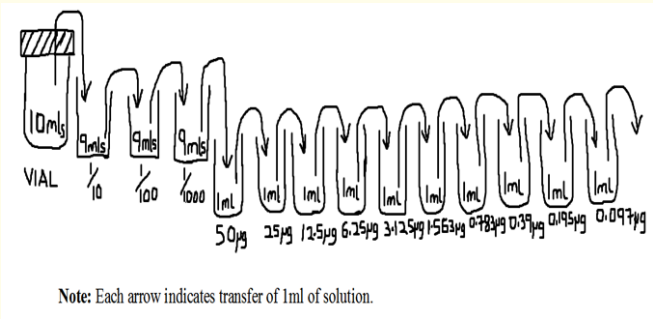

Figure 1: Illustration for the serial dilution procedure used for brands 'A' and 'B'.

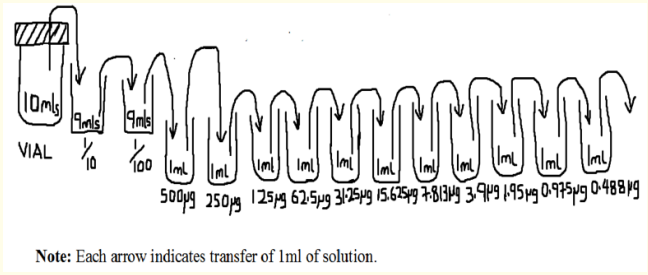

Figure 2: Illustration for the serial dilution procedure used for brands 'C', 'D', 'E', 'F', 'G', 'H', 'I', 'J' and 'K'.

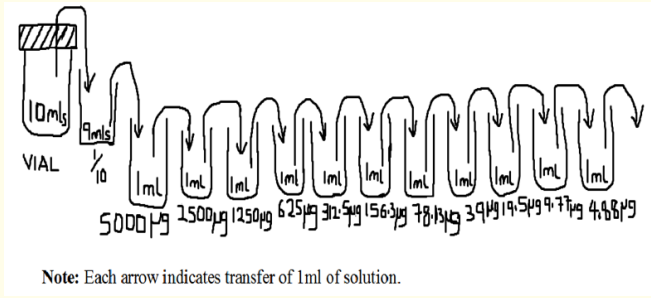

Figure 3: Illustration for the serial dilution procedure used for brands 'J' and 'H'.

The limit of quantification of the antibiotic MIC was defined by visual inspection as the smallest concentration of drug that produced a clearly distinguishable inhibition of the respective bacteria while the MBC of a given brand was taken as the lowest concentration of that brand that did not allow bacterial growth (killed the bacteria).

Determination of MBC

All tubes not showing visible bacterial growth (or turbidity) were sub-cultured on to the solid sterile medium (each sample was aseptically inoculated into a different Muller-Hinton(MH) agar plates by spread plate method using a sterilized wire loop) and incubated at 37^0C for a duration of 24 to 48 hours. Growth was determined by the presence of visible bacterial colonies (characteristic to the inoculated bacteria) on MH agar. The MBC was given by the concentration in the tube with the lowest dilution of the drug that did not show growth on MH agar.

Experimental controls: The following controls below were set together with the samples;

- A sample of the different brands of the reconstituted ceftriaxone at the usable concentration, were incubated to test for sterility.
- A bottle of sterile medium was incubated, to test for sterility.
- A bottle of the medium with viable bacteria was incubated to test for its ability to support bacterial growth.
- The standard bacteria was sub-cultured on a solid medium and incubated to show that the bacteria are actually capable of multiplying.

All the above controls were incubated simultaneously with the other test tubes containing the test solutions.

The colonies that were formed after sub-culturing were characteristic of *Staphylococcus aureus* and *Escherichia coli* respectively. The MIC and MBC values of the different brands of ceftriaxone obtained were compared with the MIC and MBC values of the reference brand [20]. Generally, the subculture results showed either no growth of microbes (indicating that the whole bacterial inoculum has been killed) or reduced number of colonies as compared with the sample from the control tube (indicating partial or slow bactericidal activity).

Calculation of the potency Ratio and relative potency

The assay of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of a given brand of ceftriaxone for potency was used to illustrate whether that brand on the market actually had appropriate antibiotic activity or not. The ratio of doses giving equivalent effects (potency ratio) indicates the relative potency of the two products [21]. Potency ratio was defined as the ratio of MBC of a given brand to the MBC of the reference brand [22] and calculated as a percentage in the formula below; Potency ratio = MBC value of a given ceftriaxone brand/MBC value of the reference ceftriaxone brand.

Results

Eleven brands of ceftriaxone (coded A, B, C, D, E, F, G, H, I, J, K) were identified and analyzed. Nine (A, B, D, E, F, G, H, I, and J) of the brands were obtained from nine drug outlets (three pharmacies and one drug shop), while two brands (C and K) were obtained from the regional referral hospital. All the samples had the same label claim of the strength of one gram. Brand 'A' was the used as a reference ceftriaxone brand for this study. Only brands 'A' and 'B' were manufactured from Europe. All the other brands were manufactured in Asia (99% from India).

The dissolution period for brands were manufactured in Asia was much longer (40-180 seconds) and was even worse for two of these brands ('I' and 'F') at three to five minutes compared those manufactured in Europe (10-15 seconds).

MIC of the ceftriaxone brands

Against *Staphylococcus aureus,* brand 'G' had the highest MIC values (31.25 µg) while brands 'A' and 'B' had the lowest MIC values (3.13 µg and 1.56 µg respectively). Brands 'A' and 'B' exhibited the lowest MIC (1.56 µg and 1.56 µg respectively) while Brands 'C' and 'H' had the highest MIC values (62.5 µg and 62.5 µg respectively). Details are illustrated in the figure 4.

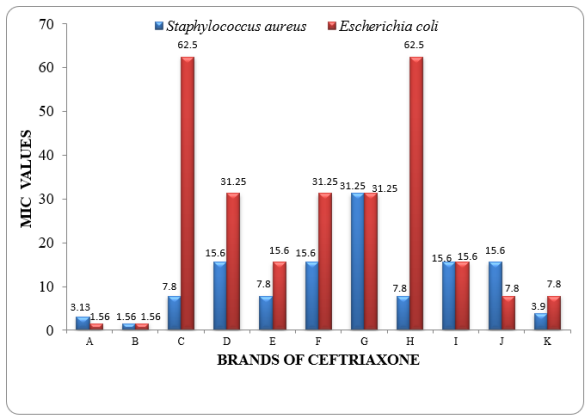

Figure 4: A graph showing MICs of the different ceftriaxone Brands analyzed.

Six of the brands ('A', 'B', 'C', 'E', 'H' and 'K') had their MIC values below 10 µg with the other five brands ('D', 'F', 'I', 'J', and 'K') having MICs above 10 µg. Similarly, four brands ('A', 'B', 'J', and 'K') had their MIC values below 10 µg while the other seven brands had their MICs above 10 µg, against *Escherichia coli*.

Brand 'G' had the highest MIC value against *Staphylococcus aureus*, which was about ten times that of the reference brand. This means that one required a big magnitude of "step-up doses" of brand 'G' (about ten times) to attain comparable inhibition effects of the reference brand. On the other hand, brands 'A' and 'B' exhibited the highest bacterial inhibitory activity (lowest MIC values) while brands 'C' and 'H' had the lowest bacterial inhibitory activity (highest MIC value) with substantially inferior inhibitory activity against *Escherichia coli* compared to the reference brand.

MBC of ceftriaxone brands

Brand 'A' had the lowest MBC value (12.5 µg), hence it was the most potent brand against *Staphylococcus aureus*, sequentially followed by brands 'B' (25 µg), 'E' (31.2 5 µg), 'K' (31.25 µg) and 'G' (62.5 µg). On the other hand, brand 'J' exhibited the highest MBC value (312.5 µg), indicating that it exhibited the lowest potency against *Staphylococcus aureus* among all the brands. Against *Escherichia coli,* brands 'A' and 'B' exhibited the lowest MBC values (6.3 µg) indicating that they were the most potent brands, sequentially followed by brand 'E', brand 'D' and brand 'C'. Details are illustrated in figure 5.

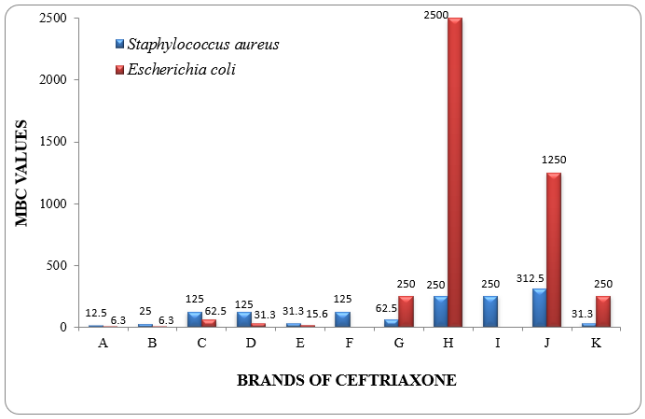

Figure 5: A graph showing comparison of MBC values of the Ceftriaxone brands.

Brands 'G' and 'K' had their MBC values at 250 µg each against *Escherichia coli*. Brands 'J' and 'H', exhibited the lowest MBC values of 1250 µg and 2500 µg respectively, hence the lowest potency among all the brands. Six brands had their MBC values above 100 µg meaning that a substantial increase in the magnitude of the administered dose (by more than tenfold) is required so as to attain a similar bactericidal effect as that of the reference brand.

The average MBC value of the ceftriaxone brands against *Staphylococcus aureus* was 122.73 µg. Brand 'J' had the highest MBC value (312.5 µg) and so was the least potent against *Staphylococcus aureus.* Thus, it exhibited the lowest bactericidal properties, sequentially followed by brands 'H' and 'I'. Implicitly, it means that brand 'J' exhibited twenty five times less bactericidal activity than the reference brand.

The average MBC of the brands against *Escherichia coli* was 485.74 µg, indicating that most of the brands had substantially low potency and are therefore expected to yield poor therapeutic outcomes when used against *Escherichia coli*.

Results of the ingeminate (repeat) experiment

The experiment was repeated for five of the brands (that had allowed bacterial growth after sub-culturing the clear solution tubes) using the serial dilution illustrated in figure 2. The experiment was not repeated for brand C against *Escherichia coli* because its' MBC value had already been determined in the earlier experiment.

Against *Escherichia coli,* brand 'J' had the lowest MIC value of 3.9 µg consequently followed by brands 'F' and 'H' while brand 'I' had the highest MIC value of 31.2 µg which is about ten times that of the brand with the lowest MIC value. Brands 'J' and 'I' had the lowest MICs of 15.6 µg against *Staphylococcus aureus,* sequentially followed by brands 'D' and 'H' while brand 'F' had the highest MIC value of 62.5 µg. This indicates that three days after reconstitution (while under refrigeration at 4^{o}C) the brands had lost a given proportion of activity and to a greater extent their potency. Results of the MIC and MBC values are shown in figure 6.

After sub-culturing these and higher concentrations of these brands which did not yield bacterial growth, on sterile plates containing Muller-hinton agar, all the concentrations of the five

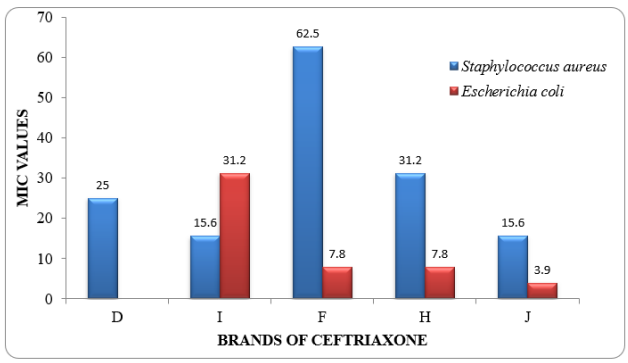

Figure 6: Comparison of MIC values for the ingeminate experiment for brands 'D', 'F', 'H', 'I', and 'J'.

brands permitted bacterial growth, hence no MBC values were noted.

Potency comparison between ceftriaxone brands having a similar shelf-life

The brands of Ceftriaxone obtained had a shelf-life of either two or three years. Comparisons of potency among the brands having the same shelf life are shown in figures 7 and 8. There were wide, non-uniform variations in the MBC values noted, also indicating wide variations in the potency of brands of the same drug with the same shelf life of two years. It is important to note that most of the brands used in the study (seven out of the eleven brands) had a shelf-life of two years.

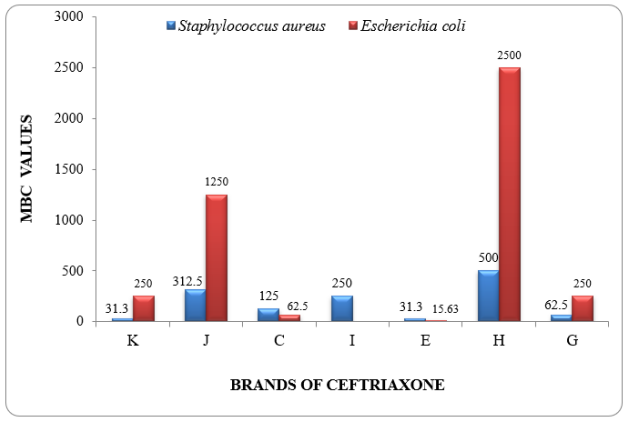

Figure 7: Potency comparisons of Ceftriaxone brands with a shelf-life of two years.

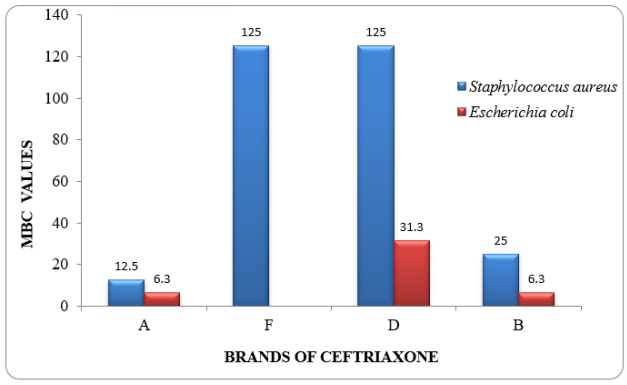

Figure 8: Comparison of potency ceftriaxone brands with a shelf-life of three years.

Figure 8 shows a comparison of MBC values of four brands of ceftriaxone with a shelf life of three years. Brand 'A' exhibited the lowest MBC values and hence, the best potency against all the standard bacteria, when compared to the other three brands in this category. Brand 'B' sequentially followed, while an exponential rise in the MBC values for brands 'D' and 'F' (about five times) was noted against *Staphylococcus aureus* and about two times, in the case of brand 'D' against *Escherichia coli.*

Comparison of the potency of Ceftriaxone brands having a similar period left to expiry

Concomitantly, the MBCs and potency of the brands with the same period (in years) left to expiry were also compared as illustrated in figure 9. There were substantial variations in the MBC values of the different brands. Despite the fact that these were ceftriaxone brands, with the same period left to expiry (in their respective categories) and are expected to be bioequivalent, they did not actually possess the same bactericidal properties. Unlike brands 'A' and 'B' which were the most potent brands against both *Staphylococcus aureus* and *Escherichia coli* among the brands of Ceftriaxone that had one year left to expiry, the other brands in this category had widely varying potency values against the respective standard bacteria.

Brand 'H' was the least potent brand as it had the highest MBC values. With one year left to expiry, brands 'A' and 'B' still had high potency (because they have low MBC values). While brands 'C' and

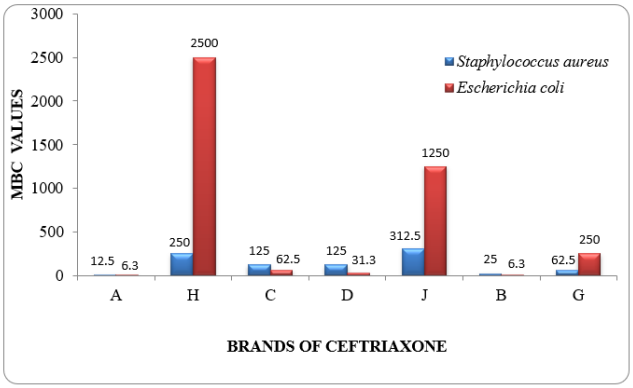

Figure 9: Comparison of MBC values and potency of ceftriaxone brands which had one year left to their expiry date.

'D' exhibited relatively diminished potency relative to brand 'A', brands 'H', 'J' and 'G' were noted to have a wide variation in their MBC values, hence had substantially lower potency against both bacteria.

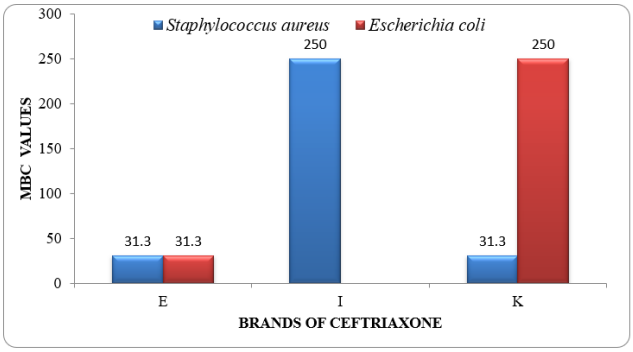

Figure 10: Comparison of potency of ceftriaxone brands which had zero years left to their expiry date.

Brand 'F' was the only brand which had two years left to expiry, and its MBC value against *Staphylococcus aureus* was 125 µg. In addition, brands 'K' and 'E' had the same MBC value (31.5 µg) against *Staphylococcus aureus* and an eightfold difference in potency against *Escherichia coli*. Likewise, as similar MBC value was noted for brand 'I' against *Staphylococcus aureus.*

Potency Ratio calculation and relative potency of the various ceftriaxone brands:

Brand 'B' exhibited the highest potency ratios among the comparator brands (0.5 and 1 against *Staphylococcus aureus* and *Escherichia coli* respectively), while brands 'H', 'I' and 'J' had the lowest potency ratios (0.05 and 1 against *Staphylococcus aureus* and *Escherichia coli* respectively) as detailed in table 1.

Brand of Ceftriaxone	Potency Ratios			
	Staphylococcus aureus		*Escherichia coli*	
	Ratio	Percentage (%)	Ratio	Percentage (%)
A	1	100	1	100
B	0.5	50	1	100
C	0.1	10	0.1	10
D	0.1	10	0.2	20
E	0.4	40	0.4	40
F	0.1	10	-	-
G	0.2	20	0.025	2.5
H	0.05	5	0.0025	0.25
I	0.05	5	-	-
J	0.04	4	0.005	0.5
K	0.4	40	0.025	2.5

Table 1: Potency ratios for the various brands of ceftriaxone.

There were no potency values for brands 'F' and 'I' obtained against *Escherichia coli* because both brands allowed bacterial growth using the serial dilution method illustrated in figure 2, after sub-culturing. However, the rest of the brands exhibited much lower potency of less than 50%, with an average potency of 16%. Three brands ('C', 'D' and 'F') had a percentage potency ratio of 10%, while even much lower percentage potency ratios of 5% (for brands 'H' and 'I') and 4% (for brand 'J') really indicated compromised bactericidal activity.

Five of the brands ('K', 'J', 'H', 'G' and 'C') had a percentage potency ratio of 10% and below, of which two brands ('G' and 'K') had 2.5% and two other brands ('J' and 'H') had a percentage potency ratio of less than 1%.

Discussion

The study evaluated the antimicrobial activity of different ceftriaxone brands by determining their potency and also made comparisons between their respective potency ratios. Generally, ceftriaxone brands exhibited widely varying anti-bacterial activity

against *Staphylococcus aureus* and *Escherichia coli*. Antibiotic activity demonstrated by the ceftriaxone concentrations from this study indicated mere inhibition of bacterial growth, which predicts a likelihood of bacterial infection recurrences in a patient, but also contribute to gradual development of drug resistance.

MIC and MBC values of ceftriaxone brands

All the brands had a relatively higher inhibitory activity against *Staphylococcus aureus* than against *Escherichia coli,* with the exception of brands 'A' and 'E' (had lower inhibitory activity against *Staphylococcus aureus* than *Escherichia coli*) and brands 'B', 'G', and I (had the same inhibitory activity against both types of bacteria). This also indicates that most brands exhibited higher anti-bacterial activity against the gram negative bacteria (*Escherichia coli*) than the gram positive bacteria (*Staphylococcus aureus*). Similarly, it was noted that a substantial increase in the administered dose would be required for the brands with MIC values above 10 µg, in order to yield the desired inhibitory effect.

On average, the brands sold on the market were ten times less active than the reference brand. MBCs were considered as the potency measure, because ceftriaxone is a bactericidal drug. According to the innovator's guidelines, the MBC values against *Staphylococcus aureus* are generally higher than those against *Escherichia coli* [20]. This was noted mainly, for the reference brand, brands 'B', 'C', 'D' and 'E' which implies that they exhibited higher potency against *Escherichia coli* than *Staphylococcus aureus*. The other brands had substantial variations in MBC values with lower MBCs and higher potency against *Staphylococcus aureus* than *Escherichia coli*.

Incidentally, against *Escherichia coli*, all the brands of ceftriaxone exhibited much lower bactericidal activity (with the exception of brand 'B', which had similar bactericidal activity like the reference brand). It implies that the dose of brand 'J' needs to be increased twenty five times to attain similar bactericidal effects of the reference brand. Likewise, the doses of brands 'H' and 'I' have to be increased twenty times to attain similar bactericidal effects of the reference brand.

Against *Escherichia coli,* brands 'J' and 'H' were of particular concern due to their very poor bactericidal properties. In addition, the very high bactericidal concentrations of the drug may not be realistically achievable since the desired plasma therapeutic concentration may never be reached as a result of the very high MBCs noted. The extremely high MBC values could be attributed to possible diminished quantity of the active ingredient in these products, hence the poor quality of the product.

Potency of ceftriaxone brands

All the brands exhibited lower potency implying low bactericidal activity and hence poor potency compared to the reference brand. This finding is consistent with findings from a similar study done in Nigeria that noted that few of the brands were sensitive, while a good number of them had lost their usefulness/effective antibacterial activity [23]. Some authors have argued that generic antibiotics behave differently from the innovator product against pathogenic microorganisms [24], which was still the case for this study where different brands of ceftriaxone yielded substantially varying results with regard to potency and efficacy.

All the ceftriaxone brands when compared with the reference brand demonstrated a substantially low bactericidal activity (with the exception of brand 'B'). This suggests poor product quality and attempts to explain the poor therapeutic outcomes in instances where these ceftriaxone brands are used, which is possible, especially if a given brand does not meet the required quality standards [25]. For instance, contaminants in generic drugs may interfere with their antibiotic activity [26]. These ultimately constitute a grave danger to health [23].

The study findings also portray that different brands of ceftriaxone are actually not pharmaceutical equivalents which is consistent with findings from a study done by Edelberto and colleagues [26], since all the ceftriaxone brands evaluated did not perform equally well (some brands did not fulfill the requirements for antimicrobial therapy). This could partially be attributed to the fact that potency of a given drug depreciates gradually during its storage period, as the active pharmaceutical ingredient in a drug is highest in the month or year of manufacture and gradually depreciates as the drug nears its' expiry date [14].

All the brands had percentage potency ratios of less than 50%, indicating that doubling the dose of the drug is expected not to kill *Escherichia coli* bacteria. These potency ratios are too low to cause bactericidal effect when the recommended therapeutic

doses for ceftriaxone are administered to a patient, even when the recommended maximum doses are administered. However, increasing the dose of brand 'E' by three times may yield sustainable therapy while the rest of the brands had less than 30% percentage potency ratio.

For all medicines especially antibiotics, quality and effectiveness are vital parameters; without them, the health of the patient is at risk. Another possible explanation for the discrepancy is that the active ingredients in these brands could have been less than what was indicated on the label claim which is a serious concern of drug safety and also has an impact on health outcomes [27]. This could result in poor therapeutic outcomes and also contribute to the development of resistance. Resistance due to over use and adulteration of the antibiotics has also been reported [23].

From this study, it was noted that the brands available on the market are more potent against *Staphylococcus aureus* than *Escherichia coli,* contrary to the expectation that ceftriaxone is more potent against *Escherichia coli* than *Staphylococcus aureus* [20]. The findings also suggest that some of the ceftriaxone sold on the market are substandard and may not have contained the required Active pharmaceutical Ingredient needed to exert adequate bactericidal or bacteriostatic activity. This is a reflection of what goes on in many developing countries particularly in sub-Saharan Africa where economically disadvantaged persons are most likely to contract communicable diseases and least likely to access quality medicines [23,28]. The substantially low potency ratios of ceftriaxone brands suggest a great risk to patients and thus caution needs to be taken to ensure patients receive quality medicines to guarantee optimum therapeutic outcomes.

Whereas a systematic review identified 11/14 (78.6%) studies that found no significant difference between the generic and innovator medicines with regard to MICs and MBCs [29]. However, most of these studies were done in countries in other continents, not in sub-Saharan Africa. Notably, the Lambert., *et al*. performed a study on the pharmaceutical quality of 34 ceftriaxone generics and found that quality standards were violated on 18 occasions, a finding which was similar to what was found in this study.

Repeat experiment and storage

When the experiment was repeated, the ceftriaxone brands generally exhibited higher MIC values against *Staphylococcus aureus* than against *Escherichia coli*. All the brands had greatly diminished inhibitory activity against *Staphylococcus aureus* when compared with MICs in the second experiment, except for brands 'J' and 'I' with substantially higher MIC values while a reverse effect was noted against *Escherichia coli* (reduction in MICs). The latter effect could have been as a result of a laboratory error. However, this findings was similar to those from a study done on Nepal on *in vitro* activity of three ceftriaxone brands against various bacteria isolates that found the MICs being different with the MIC of one of the brands being higher by at least two fold indicating lower *in vitro* antibacterial activity [30].

Despite the fact that a clinically significant loss of potency is attained when a threshold reduction of 10% from the initial concentration is registered [31], there was an even higher reduction (more than 10%) in potency of all the five brands being assayed, three days (under refrigeration at 4°C) after reconstitution evidenced by bacterial growth. Upon sub-culturing the ceftriaxone brands, the bacteria grew on all the plates having varying concentrations of the five brands assayed, indicating that no concentration was able to inhibit the growth of the bacteria). Much larger bacterial colonies were observed during the repeat experiment than in the first round. This further confirms that the five brands assayed had much lower potency. Hence, these point to characteristics of poor quality drugs whose potency was greatly reduced after three days deviating from the expected retention of potency of not less than a 10% fall/reduction, even within the recommended storage conditions.

Comparison of rates of dissolution for the different brands

Generics need to meet the standard pharmacokinetic parameters in the body (they must dissolve at the same rate and to the same extent as the innovator brand). This process ensures that the products are bioequivalent (they should behave the same in the body and should also have similar physicochemical properties). Unfortunately, it took a relatively longer period (40-180 seconds) for the complete reconstitution of the brands which were not manufactured in Europe. This could be attributed to possible gaps in current Good Manufacturing Practice (cGMP) and probable poor quality pharmaceutical products on the market in low and middle income countries to minimize costs of production [32,33]. These economic considerations should not be traded for the price of lower quality of patient care.

This study demonstrated an easy, quick and reliable way of establishing the potency of a given antibacterial product in a resource limited setting using broth dilution antimicrobial susceptibility testing [34]. However, a major limitation was that the study area may not have been representative of the entire country status. Thus, future research may be done on a larger scale, preferably, countrywide to capture all the ceftriaxone brands used countrywide and in the East African region. In addition, further investigations regarding the amount of active ingredient, chemical purity, and in-vivo activity of the ceftriaxone brands also has to be evaluated. Therapeutic equivalence studies could also provide much more information about the *in vivo* pharmacologic activity of the different ceftriaxone brands, so as to help enhance therapeutic outcomes.

Conclusion

Ceftriaxone brands on the market had widely varying level of antibacterial activity. The study findings affirm and attempt to explain the differences reported therapeutic outcomes when different ceftriaxone brands are used for treatment of ailments with bacterial origin. Generally, the reference brand and brand 'B' exhibited the highest potency (were the most potent brands) while brands 'H' and 'J' displayed the lowest (worst) potency against *Staphylococcus aureus* and *Escherichia coli*. Brands 'I' and 'F' exhibited the worst dissolution properties; however, their potency against *Escherichia coli* could not be fully established.

Healthcare facilities and staff can easily adapt this approach of evaluating potency of an antibiotic, through policy, so as to improve treatment outcomes and affordability of care. It provides health workers with a solution to the task of making a rational choice for which brand to choose from a large number of ceftriaxone brands on the market, so as to attain the best therapy outcomes. In addition, regular monitoring and assessment of the efficacies, potencies and qualities of antibiotics sold in a particular area should be done since it is critical in planning the best remedies for empirical treatment of infectious patients.

Ethics, Consent and Permissions

Ethical approval, consent and clearance for the study was sought from the Mbarara University of Science and Technology Research Ethics Committee, and the pharmacy/drug shop staff and owners.

Consent for Publication

Not applicable.

Availability of Data and Materials

All the data generated or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

AS designed and conceptualized the study, wrote the model and conducted data collection. AS conducted analyses. AS wrote the first draft of the manuscript.

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