In Vitro Comparison Of Generic And Branded Formulations Of Ceftazidime Using Standard Strain Of Pseudomonas

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Abstract: Background& Objective: Use of generic medicines has been increasing in recent years as a cost saving measure in health care provision. But, there is an uncertainty about whether the quality of a generic medicine is equal to, greater than or less than its equivalent brand-name drug. Its quality must be evaluated in vitro and in vivo in order to confirm their suitability for therapeutic use. Here, we have done in vitro comparison of generic and brand formulation of ceftazidime against pseudomonas standard strain (ATCC 27853). Methodology: One generic and three brands of ceftazidime were selected for in vitro comparison. Microbiological assays were used to establish the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against pseudomonas standard strain (ATCC 27853) according CLSI (Clinical Laboratory Standard Institutes) guidelines. Results: The MIC values of the ceftazidime samples evaluated (Brand and generics) were the same for pseudomonas standard strain tested, indicating that all products behaved similarly. The MBC values were very similar for all samples. Overall, therefore, the results showed no significant differences among samples. Conclusion: Reference method MIC and MBC testing of ceftazidime against pseudomonas has demonstrated no significant difference in in vitro activity between generic and brand products. [Tank N NJIRM 2016; 7(2):31-36]

Key Words: Ceftazidime, Pseudomonas, Generic drug, Brand drug, MIC, MBC.

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Introduction: Generic medicinal products are 'copies' of patented drugs and can be marketed at low cost following patent expiry of the brand leader preparation. The main purpose of generic drug development is to reduce the price of marketed drugs and ultimately to lower public health costs. As a consequence of increasing restrictions on the economic resources allocated to public health programmes, many governments now strongly support the production and clinical use of generic medicinal products in place of reference brand-name drugs. [1] In India also, under The Jan Aushadhi Campaign launched in 2008 more than 45 generic stores are started to ensure quality medicines under reasonable prices. [2] Though generic drugs contain the same active ingredients as the original brand name drug but there is an uncertainty about whether the quality of a generic medicine is equal to, greater than or less than its equivalent brand-name drug. Its quality must be evaluated in vitro and in vivo in order to confirm their suitability for therapeutic use.

Here, we have done in vitro comparison between generic and brand formulation of ceftazidime against pseudomonas standard strain and evaluated that no difference in antimicrobial activity between them.

Material and Methods: Study Site: Study was carried out at Microbiology Department of P.D.U. Govt. Medical College, Rajkot.

Drug samples: The generic sample of ceftazidime 1 gm vial was taken from the government hospital. The marketed three brands of ceftazidime 1 gm vials were taken from retail pharmacy stores. The samples obtained were stored according to the manufacturer's packaging instructions and kept there until testing. Samples were coded 1 to 4 (1- brand 1, 2- brand 2, 3- generic, 4- brand 3) and the microbiologist who conducted the study was kept blinded.

Bacterial strain: Standard strain of Pseudomonas (ATCC 27853) was used in the study according to CLSI guidelines. [3]

Microbiological assay: First to ensure quality standard of strain (ATCC 27853), it was tested for ceftazidime sensitivity by Kirby Bauer disc diffusion method. [3]

Minimum Inhibitory Concentration (MIC) of ceftazidime for pseudomonas was tested by Agar dilution method in accordance to CLSI guidelines. [3] First, 10 ml of sterile water was added to all 4 vials of 1 gm ceftazidime, so strength of the resulting solution was 100 mg ml^{-1}. Then serial dilution of all 4 ceftazidime from 100 mg ml^{-1} to 640 µg ml^{-1}(100 mg ml^{-1}, 10 mg ml^{-1}, 1 mg ml^{-1} then taking 6.4 ml of 1 mg ml^{-1} + 3.6 ml DW) was made in wells. Then taking 2ml (from 640 µg ml^{-1}) + 18 ml Muller Hinton Agar, so final concentration of 64 µg ml^{-1} achieved. So on, 32µg ml^{-1}, 16µg ml^{-1}, 8µg ml^{-1}, 4µg ml^{-1}, 2µg ml^{-1}, and 1µg ml^{-1}.

We had prepared standard McFarland solution which contained 1.5×10^8 bacteria colony and diluted it ten times. Then 2µl from this colony was dispensed on each agar plate. It was incubated at 37° c for 16-24 hours and the growth was observed. Lowest concentration of the Ceftazidime which completely inhibits the growth on agar plates was considered as Minimum inhibitory concentration [MIC].

To check standard Sterility control (MHA without drug) and Quality control (MHA with drug) were also incubated.

To check MIC, we had also done broth dilution method according to CLSI guidelines. [3] We had diluted drug concentration of 640 µg ml^{-1} five times, so concentration of 128 µg ml^{-1} was achieved. Then by adding nutrient broth we had double diluted it & got concentration of 64 µg ml^{-1}, 32 µg ml^{-1}, 16 µg ml^{-1}, 8 µg ml^{-1}, 4 µg ml^{-1} and 2 µg ml^{-1}. We had prepared standard McFarland solution 1:150, which contained 1×10^6 bacteria colony per ml. Then we had diluted 1 ml of McFarland solution to each well containing 1 ml of drug in dilution series. Finally, a 1:2 dilution of each drug concentration and a 1:2 dilution of bacteria colony were done. All wells were incubated at 37° c for 16-24 hours and growths in the wells were assessed with naked eye examination. MIC was defined as the lowest dilution that showed no visible growth (turbidity).

To check quality standard, in 1st well positive control (broth + bacteria without drug), 2nd well negative control (broth + bacteria with drug) were also incubated.

To measure Minimum Bactericidal concentration (MBC), sub cultures of all the dilution that showed no visible turbidity, of all 4 samples of ceftazidime were done on Mac conkey agar and incubated at 37° c for 24 hours. MBC was defined as the lowest dilution that showed no growth on culture.

Costs of all samples of ceftazidime were also analyzed.
The Animal Welfare and Ethical statement:

As this was an in vitro study using standard strain of pseudomonas (ATCC 27853) there was no need to require prior approval by Institutional Animal Ethics Committee.

Results: By Kirby Bauer disc diffusion method, zone of inhibition by ceftazidime 30 microgram disc on standard strain of Pseudomonas (ATCC 27853) [Fig. 1] was 24 mm.

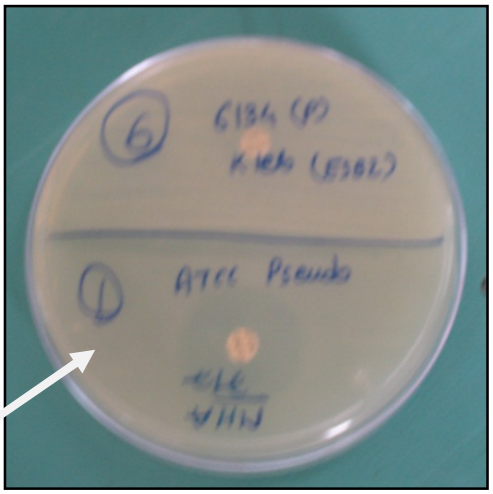

Figure 1: Lower half of disc showing Zone of inhibition by 30µg disc of ceftazidime on ATCC 27853 strain of pseudomonas by Kirby Bauer Disc diffusion method.

Arrow shows zone of inhibition.

After 24 hours incubation at 37°c, in agar dilution method there was no growth on any agar plate at different drug dilutions of 64 µg ml^{-1} up to 1 µg ml^{-1}. So, MIC of all 4 samples was considered same (<1 µg ml^{-1}). [Fig. 2]

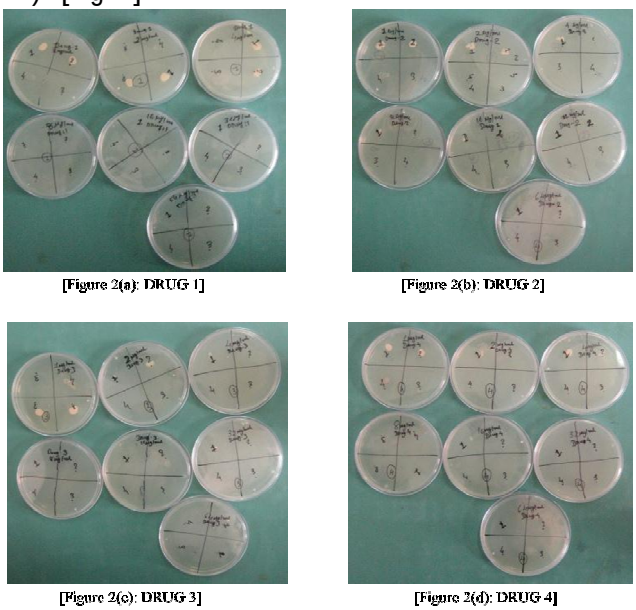

Figure 2: Part of agar plates with written no 3 on them, showing result of MIC of ceftazidime on ATCC 27853 strain of pseudomonas by Agar dilution method of all

4 drugs at concentration of 64 µg ml^{-1} up to 1 µg ml^{-1}. Lowest concentration of drugs which show no growth on plate with naked eye examination considered as MIC. (MIC, Minimum Inhibitory Concentration)

Sterility control remains unaltered and there was no growth seen in Quality control.

By naked eye examination in broth dilution method, no growth (turbidity) was seen in any well of samples 1, 2 and 3 at serial dilutions from 32 µg ml^{-1} up to 1 µg ml^{-1} except drug 4 which showed growth (turbidity) at 1 µg ml^{-1}. [Fig. 3] But the difference in MIC of sample 4 compared to others (sample 1, 2 & 3) was not significant and it was also in range of sensitivity according to CLSI guidelines. So, MIC of all 4 samples was same.

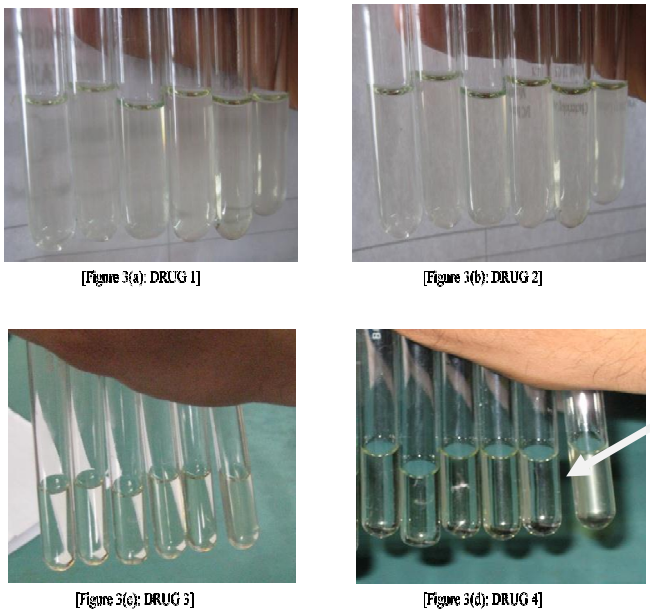

Figure 3 showing result of MIC of ceftazidime on ATCC 27853 strain of pseudomonas by Broth dilution method of all 4 drugs at concentration of 32 µg ml^{-1} up to 1 µg ml^{-1} (concentration in decreasing order from right to left). Lowest concentration of drugs which show no turbidity in wells with naked eye examination considered as MIC. In fig.III (d) drug 4 shows turbidity at concentration of 1 µg ml^{-1} which is indicated by arrow. (MIC, Minimum Inhibitory Concentration)
Positive control showed gross turbidity and no growth was seen in negative control.

After 24 hours incubation of culture on Mac conkey agar at 37°C, MBC were assessed for 1 µg ml^{-1} & 2 µg ml^{-1} dilutions of all samples. It showed that there was no growth for any sample (1, 2 & 3) except sample 4 which showed growth at 1 µg ml^{-1}. [Fig. 4] But it MBC was in range of sensitive according to CLSI guideline & the difference in its MBC to others (sample 1, 2 & 3) was not significant. So, MBC of all 4 samples we had considered same.

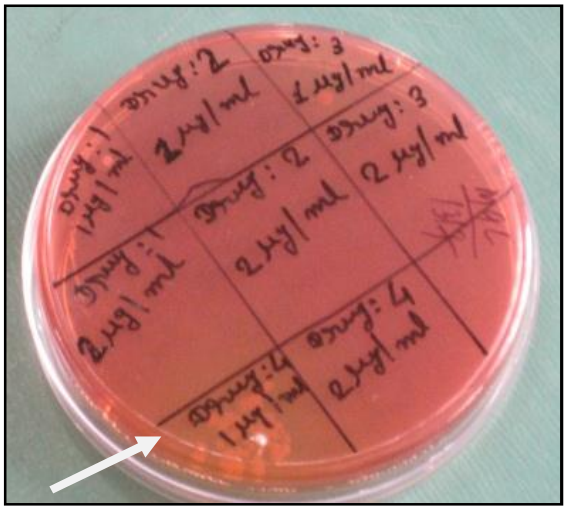

Figure 4: Showing result of MBC of ceftazidime by culture on Mac conkey agar of all 4 drugs at concentration of 2 µg ml^{-1} to 1 µg ml^{-1}. Lowest concentration of drugs which show no growth on culture considered as MBC. In figure drug 4 at concentration of 1 µg ml^{-1} shows growth which is indicated by arrow. (MBC, Minimum Bactericidal Concentration)

According to study design, only evaluated difference between them was their cost. It was considerably higher for brand samples in comparison to generic sample. [Table 1]

Table 1: Showing cost difference between generic and brand samples of ceftazidime.

Coding	Samples	Cost in Rs. (1 gm vial)
1	Brand 1	255
2	Brand 2	253
3	Generic	32
4	Brand 4	143

The MIC is an in vitro microbiological assay used worldwide to determine the susceptibilities of micro-organisms to particular agents. The decision on whether or not to use a particular antimicrobial agent is based on the information derived from MICs. MBC testing is not routinely done, but we thought it would

be useful to evaluate in vitro comparison. [4] It would therefore seem prudent to evaluate a generic and brands products using this same platform that is used daily in clinical microbiology laboratories. Comparing the MIC of the generic and its brands is indicative of in vitro efficacy. [5]

A small difference between MIC and MBC indicates that antibiotic is primarily bactericidal, while a large difference indicates bacteriostatic action. Ceftazidime belongs to cephalosporin group of antibiotic which is primarily bactericidal. [6] Here, a small or no difference between MIC and MBC result of all samples favours the cidal action of ceftazidime.

Discussion: The use of generic drugs is indicated from many countries in order to reduce medication price.[7] According to WHO, a generic drug is a pharmaceutical product, usually intended to be interchangeable with an innovator product that is manufactured without a license from the innovator company and marketed after the expiry date of the patent or other exclusive rights.[8] Generic drugs are equivalent to brand name drugs in their bioavailability and composition as well as strength, route of administration and effects. Brand name drugs have exclusive names on which the manufacturing pharmaceutical company holds patent and the name cannot be copied by the other drug manufacturers. [9]

The difference between brand and generic drugs is only a minor one. As the brand name Drugs Company has already spent considerable time in research and trials, generics companies are not required to conduct any clinical research. Costs of generic drugs are lower than brand name drugs. The price of a brand name drug may be three times higher than its generic equivalents. Brand name drugs are produced by only that pharmaceutical company that holds its patent whereas generics are produced by many companies. Although generic drugs contain the same active ingredients as the original brand name drug, there can be slight differences in the inactive additives or fillers. [9]

Antibiotics, being the wonder drug, are widely prescribed in the developing countries. In order to produce expected therapeutic effect, the drug needs to be safe, effective and of good quality. The Governmental institutes in India, making bulk purchases of generic medicines through tender system and settling for the lowest bids. The main problem in India is that we lack quality infrastructure where the prices are controlled but quality remains unmonitored. Ineffective legal and regulatory frameworks, on-going gaps between legislation and practices on the ground and lack of transparency have led to the creeping of substandard drug into the Indian market. Another main public health concern related to antimicrobial drug is the resistance, "Super bug", on use of substandard drugs. The lives of individual patients are put at risk by substandard pharmaceutical preparations. Therefore, it is necessary to check quality of generic drugs. [10] The outcome of the present study will help raise awareness among both the government and public regarding the use of quality products.

Tested organism, *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause a wide range of infections, especially in immunocompromised people and people with severe burns, diabetes mellitus or cystic fibrosis. *P. aeruginosa* is relatively resistant to many antibiotics but effective antibiotics include imipenem, meropenem, ceftazidime, ciprofloxacin and gentamicin. [11] Ceftazidime is a semi synthetic, broad-spectrum, beta-lactam antibiotic (3rd generation cephalosporin) for parenteral administration. [12]

According to CLSI guidelines, by Kirby Bauer disc diffusion method, with 30 microgram disc of ceftazidime zone of inhibition diameter on P. aeruginosa standard strain (ATCC 27853) was interpreted as ≥18 mm: sensitive, 15-17 mm: intermediate, ≤14 mm: resistant. [3] Here, in this study First to ensure quality standard of strain, standard strain of Pseudomonas was tested for ceftazidime disc which shows zone of inhibition was 24 mm, so strain was sensitive to testing drug.

MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. [17] According to CLSI guidelines, by, MIC result of ceftazidime for pseudomonas was interpreted as ≤8 μg ml^{-1}: sensitive, 16 μg ml^{-1}: intermediate, ≥32 μg ml^{-1}: resistant. [3] In this study for all samples (generic & brands) of ceftazidime MIC result was ≤8 μg ml^{-1}, so they were sensitive. Also, MIC results were same for all so there was no difference between them. [Table 2 and 3]

Table 2: Showing result of ceftazidime MIC (in µg ml^{-1}) against standard strain of pseudomonas by Agar dilution method. (MIC, Minimum Inhibitory Concentration)

Dilution (in µg ml^{-1})	Growth on agar plate seen or not			
	Drug 1	Drug 2	Drug 3	Drug 4
64	no	no	no	no
32	no	no	no	no
16	no	no	no	no
8	no	no	no	no
4	no	no	no	no
2	no	no	no	no
1	no	no	no	no
MIC result	<1µg ml^{-1}	<1 µg ml^{-1}	<1 µg ml^{-1}	<1 µg ml^{-1}

Table 3: Showing result of ceftazidime MIC (in µg ml^{-1}) against standard strain of pseudomonas by Broth dilution method. (MIC, Minimum Inhibitory Concentration)

Dilution (in µg ml^{-1})	Growth in wells seen or not on naked eye examination			
	Drug 1	Drug 2	Drug 3	Drug 4
32	no	no	no	no
16	no	no	no	no
8	no	no	no	no
4	no	no	no	no
2	no	no	no	no
1	no	no	no	Yes
MIC result	<1 µg ml^{-1}	<1 µg ml^{-1}	<1 µg ml^{-1}	Between 1-2 µg ml^{-1}

MBC is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media. [17] In other words, it is the concentration of the antibiotic which kills 99.9% of the bacteria. [6] In this study, all 4 samples of ceftazidime had similar result of MBC showing no difference between them. [Table 4]

Table 4: Showing result of ceftazidime MBC (in µg ml^{-1}) against standard strain of pseudomonas by culture on Mac conkey agar. (MBC, Minimum Bactericidal Concentration)

Dilution (in µg ml^{-1})	Growth on culture seen or not			
	Drug 1	Drug 2	Drug 3	Drug 4
2	no	no	no	no
1	no	no	no	Yes
MBC result	<1 µg ml^{-1}	<1 µg ml^{-1}	<1 µg ml^{-1}	Between 1-2 µg ml^{-1}

Literature search also showed in vitro comparison studies which were supporting to our study result of no difference between generic and brand drug antimicrobial activity. [4, 5, 13, 14]

It has been proposed that generic antibiotics behave differently from brand products against pathogenic microorganisms.[15,16] This is possible if the generic antibiotic does not fulfil the quality standards for that pharmaceutical product (e.g., purity or content).[14] Doubts about the efficacy of generic antibiotics, arising from complaints from the medical community have been reported in the literature and at international meetings.[13] Taking into account the controversy and reports of inferior quality of some generic antimicrobial agents, comparative MIC determination serves as a basis for their initial evaluation.

Also, high cost of brand drugs in comparison to generic drug, increasing expense without real benefit. [13] Cost was approximately 8 times higher for brand 1 & 2 and 4.5 times higher for brand 3 as compared to their generic counterpart.

Not all ceftazidime brands available were analyzed. However, being a laboratory-based evaluation, it is not devoid of limitations, and it addresses neither pharmacodynamics nor pharmacokinetic issues pertaining to a particular agent. [5] Literature search has showed that in vivo studies documented that a difference in excipients is related with the loss of response and adverse effect during treatment with the generic formulation. [7] On the basis of in vitro testing we couldn't explain role of excipients that could influence gastrointestinal transit, absorption, *in vivo* solubility or *in vivo* stability of the active substance.

The lack of efficient bioequivalence methods for locally acting drugs has limited the availability of generic drugs in this category which includes inhalation, topical dermatological, nasal, optic and otic products. So, in our opinion this should be area of future research.

Conclusion: Our study result showed that there was no difference between generic and brand drugs in, in vitro antimicrobial activity, this might help to change the false belief of many public, some doctors and pharmacists who believe that "the more expensive the product, the more effective". Cost of generic drug was

so much low which favours the use of generic drug, particularly in developing countries.

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