ejpmr, 2021,8(8), 52-58.

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

BIOPHARMACEUTICAL AND MICROBIOLOGICAL EVALUATION OF SOME CEFTRIAXONE-SULBACTAM PRODUCTS FOR SAFETY AND EFFICACY

[1]*Dr. Sunday O. Awofisayo, [2] Nse Eyen, [3]Dr. Ayodeji A. Agboke and [4]Prof. Matthew I. Arhewoh

[1]Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, university of Uyo, Nigeria.
[2]Department of Food Technology, Akwa Ibom State Polytechnic, Ikot Osurua, Akwa Ibom State, Nigeria.
[3]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Uyo.
[4]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

***Corresponding Author: Dr. Sunday O. Awofisayo**

Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, university of Uyo, Nigeria.

Article Received on 02/06/2021 **Article Revised on 23/06/2021** **Article Accepted on 13/07/2021**

ABSTRACT

Background: The concept of β-lactam antibiotics in combination with β-lactamase inhibitor to combat β-lactamase producing organism is a scientific norm for therapeutic success. This study seeks to compare some biopharmaceutical and microbiological parameters of selected ceftriaxone-sulbactam brands available in Nigeria with Rocephin®, as standard, with respect to efficacy and safety. **Materials and Methods:** Physical, physicochemical and microbiological tests for quality evaluation were performed alongside clinical / microbiological evaluations using typed and clinical isolates as indices for efficacy. The tested brands were adjudged satisfactory with respect to labeling and drug presentation. Two samples, SB-C and SB-D, revealed chemical contents lower than 90% for either ceftriaxone and/or sulbactam. However, all samples revealed satisfactory and similar microbiological safety and efficacy characteristics. **Results:** Products SB-A, SB-B, SB-E, SB-F and SB-G demonstrated significantly higher antibacterial activity than the standard, ($p<0.05$). Samples SB-C and SB-D were not chemically equivalent to the other generic products of ceftriaxone-sulbactam evaluated. **Conclusion:** The presentation of ceftriaxone with β-lactamase inhibitor demonstrated similar biopharmaceutical properties but superior antimicrobial properties/ efficacy than the standard.

KEYWORDS: Ceftriaxone-sulbactam, biopharmaceutics, microbiology, safety, efficacy.

INTRODUCTION

In recent times, drugs with same label claims are produced by different manufacturers and registered for sale by the regulatory authorities.[1] These products are required to have uniformity with respect to standards of efficacy and safety. Biopharmaceutics therefore relates the physicochemical properties of drugs in their dosage form with the pharmacology, toxicology or clinical responses observed.[2] B-lactam antibiotics are classes of broad-spectrum antibiotics consisting of agents that contain a β-lactam ring in their molecular structures.[3, 4] This class of drugs is commonly presented with β-lactamase inhibitors to combat peculiar organisms capable of inactivating drugs with β-lactam ring in their molecule. Several drug products of this combination are widely used in clinical setting in the study area. Antibacterial resistance has reached an alarming level with major pathogens (including Gram-negative organisms such as *Klebsiella pneumonia*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) quickly evolving towards pan-drug resistance phenotypes. Without an adequate response to this medical issue, the burden and mortality associated with infectious diseases, and especially hospital acquired bacterial infections, is consequently expected to significantly increase in the near future.[5] There is however, scarce information on the comparative assessments of the physicochemical alongside microbiological properties of ceftriaxone sulbactam products in use in the study area.

The physical and chemical properties of a drug in its dosage form (drug product) and the route of administration have been extensively reported to affect the rate and extent of systemic drug absorption. This ultimately determines the safety and efficacy profile. The interchangeability and bioequivalence of ceftriaxone-sulbactam products hinge on the pharmacokinetic and pharmacodynamic profile which fundamentally revolves around the inherent physicochemical properties alongside formulation factors.

Ceftriaxone-sulbactam products are marketed as parenterals for intramuscular and intravenous administration. Ceftriaxone, the main drug is widely used for the treatment of gram-positive and gram-negative bacterial infections. Sulbactam is a chemical molecule

that is administered with ceftriaxone to combat the effect of β-lactamase. Sulbactam is an irreversible inhibitor of β-lactamase. It is able to inhibit the most common forms of β-lactamase encountered in clinical setting.[6]

AIM OF THE STUDY

This study was aimed at assessing the biopharmaceutical and microbiological indices for some selected parenteral ceftriaxone-sulbactam antibiotic products for safety and efficacy.

MATERIALS AND METHODS

Materials

Samples of ceftriaxone-sulbactam (CTX-SBT) products were obtained from the study area. The branded/innovator product was similarly purchased from a licensed drug distribution outlet in Uyo, Akwa-Ibom state Nigeria. The details of the drug products are listed in Table I. Clinical isolates were obtained from three (3) medical centres in Uyo metropolis from cultures obtained from different patients with respiratory and urinary infections. Isolates were identified with Vitek (bioMerieux, Inc., Hazelwood, Mo) and supplemented by conventional methods as required. Isolates were stored as suspensions until use. Dey and Engley (D/E) neutralizing agar (prepared by suspending 54.02 g in 1L of purified distilled water and heated to boiling and dissolved completely) (product of HiMedia laboratories). Epsilometer testing (E-testing) strips were manually produced from filter paper according to the method of Sanchez and Jones (1992).[7]

Table 1: Ceftriaxone-based products employed for analysis.

BRANDS/CODES	PRODUCTS DESCRIPTION			
	Product Name	**NAFDAC Registration**	**Country of Production**	**MD/ED**
Control	Rocephin	Yes	France	11-17/11-20
SB-A	Nirizone	Yes	India	03-17/02-19
SB-B	Tandak	Yes	India	11-17/10-19
SB-C	Syceph-SB	Yes	India	12-17/11-20
SB-D	Troxone	Yes	India	07-17/06-20
SB-E	Mega-2D	Yes	India	03-18/02-20
SB-F	Kingcef	Yes	China	05-17/05-20

NB: NAFDAC represents National Agency for Food Administration and Control; MD/ED represents Manufacturing date and Expiry Date. [*a] Average unit price at the remote retail outlet in the study area.

METHODS

Physical assessment of drug packaging and drug powder

Melting point determination

A sufficient amount of sample was deposited into the open end of a capillary tube up to about 2 mm loading, with the other end sealed. This was inserted into the melting point apparatus. The apparatus was turned on to heat at a medium rate of 20^{o}C/min at first determination. A fresh sample was loaded, and the determination repeated with an adjustment to a rate of 1^{o}C per min as the first determined melting point value is approached.

Uniformity of dosage units (Weight uniformity)

The content of 10 vials for each of the different products was ascertained by weighing the entire vial and subsequently emptying the powder content and vials reweighed. The difference in weight represents the weight of the content. The mean ± SD was determined for each product sample.

Pharmaceutical Quality Assessment

Colour of vial content

The colour of the powder was assessed visually by examining a small quantity of powder placed on a flat white tile and then black background/surface. The colour was matched with colour chart by 5 assessors. Each assessor evaluated the products individually and their observations were considered objectively. The consistent decision of at least two of the assessors was taken.

Clarity of solution

Samples of 10% w/v (100 mg in 10 ml injection water) were prepared. The assessors similarly observed the reconstituted drug against a white and then a black background under sufficient illumination. The consistent decision of at least two of the assessors was taken.

Colour of reconstituted solution

The color of the reconstituted solution was assessed by examining a 10% w/v aqueous solution in a clean colourless test tube placed against a white background. The assessors similarly observed the reconstituted drug. The consistent decision of at least two of the assessors was taken.

Content uniformity

The chemical content (ceftriaxone and sulbactam was determined by high pressure liquid chromatographic assessment using the method of Shrivastata and coworkers.[8] Chromatographic separation was achieved on Agilent 1200 series system equipped with G1311A quartenary pump and ODS Hypersil C-18 column (250mm x 4.6 mm, 5µm). The system was attached to Agilent variable UV/Vis detector set at 220 nm. The mobile phase consisted of a binary mixture of

acetonitrile and tetrabutylammonium hydroxide (TBAH) adjusted to pH 7 with orthophosphoric acid at ratio 70:30. The injection volume was 10 µl and run time was 15 min. The determination was performed at room temperature, 25^{o}C.

Microbiological test

Disk diffusion in test

Prior to testing, the isolates were inoculated on potato dextrose agar (Remel, Lenexa, Kans) and chromogenic isolation medium, CHROMagar (Hardy Laboratories, Santa Monica, Calif.). One Mueller-Hinton (MH) agar plate was used for each organism tested. The MH agar plates were allowed to come to room temperature and appropriately labeled. The inoculums of the samples were prepared according to standard protocols in saline. With the use of a sterile inoculating loop, the inoculums were taken and streaked on the MH agar in a back and forth motion. Antibacterial discs for the different samples were placed on the agar using a forceps, one at a time and subsequently placed in a 35 ± 2°C incubator for 24 h. The zone of inhibition for each drug product against the organisms were measured comparing with standard Clinical Laboratory Standard Institute (CLSI) guidelines as resistant, intermediate or susceptible. Antibiotic susceptibility assay was carried out using.[9, 10]

E-Test (Diffusion and dilution)

The strips were placed on an inoculated agar plate, for an immediate release of the impregnated drug. An overnight incubation was observed to produce a symmetrical inhibition ellipse. The minimum inhibitory concentration (MIC) value over a wide concentration range (> 10 dilutions) was determined by the intersection of the lower part of the ellipse-shaped growth inhibition area with the test strip.

Biological tests

Sterility

Percentage strength of 15% w/v (CTX-SBT) was prepared for the drug samples by dissolving the entire vial content in 10 ml of sterile water for injection, for each of the brands. Three vials were tested (n=3). The vials content was dissolved in the sterile water for injection provided with the pack. The antimicrobial activity of the drug solution was neutralized by dilution in a sufficient quantity of recovery broth D/E neutralizing broth. As such, concentrated culture media (i.e. double strength) were used to accommodate the ensuing dilution. Two culture media; double strength plate count agar (PCA) and double strength sabouraud dextrose agar (SDA), were used for the test. A volume of 0.1 ml of drug solution was transferred into molten double strength PCA and another 0.1 ml into molten double strength SDA. These were then transferred into sterile Petri dishes, allowed to solidify, inverted and incubated (PCA at 35-37°C and SDA at 25°C). These were repeated for all brands. Microbial growth on the incubated culture media was checked after 3, 7, and 14 days of incubation.

Bacterial endotoxin-pyrogen test

The gel-clot technique was employed based on gel formation and the quantities of endotoxin are expressed in USP Endotoxin Units (one IU of endotoxin = one USP-EU).

a. Preparation of standard endotoxin stock solution and standard solution
   The entire content was reconstituted (1 vial of the sample with 5ml of Limulus Amoebocyte Lysate (LAL) reagent water, mixed intermittently for 30 min, using a vortex mixer. The concentrate was used to make appropriate serial dilutions. Some part of the concentrate was reserved in a refrigerator for subsequent dilutions.
b. Preparation of sample solution and determination of maximum valid dilution
   Sample solutions were prepared by dissolving vial content for each sample using LAL Reagent water. The pH of solution was adjusted so that the pH of the mixture of LAL Reagent and sample falls in the pH range specified by the LAL manufacturer. The maximum allowable dilution (MVD) for the samples at which the endotoxin limit can be determined was calculated.

$$MVD = (Endotoxin\ limit \times \frac{Concentration\ of\ sample\ solution}{\gamma})$$

…….Equation 1

Statistical analyses

Statistical analyses were performed using the R- 4.0.2 for Windows statistical computing (R Core Team, Vienna, Austria). Welch t-test was used to analyze categorical variables. A two-tailed *P* value of 0.05 was considered statistically significant.

RESULT

Physicochemical characteristics

The physical characteristics of the samples employed in the study are listed in Table II. The powder colour and texture were observed and outcome as presented. All the drug samples revealed light yellow coloured powders and on reconstitution produced clear solutions. The physicochemical parameters of the samples are presented in Table III. The parameters include average weight of vial content, melting point of powder, pH of reconstituted solution and chemical content for ceftriaxone and sulbactam are presented for each sample. Some of the samples failed the chemical content tests as they did not comply with the BP specification of not less than 90% and not more than 110% of active ingredient.[11] Sample SB-C and SB-D revealed lower ceftriaxone and sulbactam contents while sample SB-A had lower sulbactam content. Table IV reveals the bacterial endotoxin test outcome for samples. All samples passed the gel clot and chromogenic tests.

Antibacterial susceptibility test on the samples are presented in Table V. The respective MIC values for the samples against typed bacterial strains and clinical isolates. The result revealed significant differences

within the samples on account of the MIC values. The test samples produced significantly higher antibacterial activity compared to the standard against typed strains of bacteria, (i.e, *E. coli* NCTC 10418 (P=0.003), *P. aeruginosa* 10662 P=0.0001), *S. aureus* NCTC 6571 (P=0.0001), and *H. influenza* ATCC 40247 (P=0.0001). Similarly, on the clinical isolate, test samples revealed significantly higher antibacterial activity against *Streptococcus spp.* (P=0.0001), *Neisseria spp*. (P=0.0117), *Pseudomonas spp*. (P=0.0001) and *Staphylococcus spp* (P=0.0001). On the contrary, there was no significant difference in the test samples compared with the standard against *Haemophilus influenza* (P=06279).

Table II: Physical assessment of ceftriaxone-sulbactam products.

SAMPLE CODES	PRODUCT DESCRIPTION	
	Powder colour	**Powder texture**
Control	Off white	Smooth
SB-A	Cream	Gritty
SB-B	Cream	Smooth
SB-C	Off white	Coarse
SB-D	Cream	Smooth
SB-E	Cream	Smooth
SB-F	Cream	Coarse
SB-G	Cream	Smooth

Table III: Physicochemical Assessment.

SAMPLE CODES	PHYSICAL ASSESSMENT OF INJECTION POWDER AND RECONSTITUTED DRUGS			CHEMICAL CONTENT (N=3) (%W/W)		
	Average weight of powder (g ±SD)	**pH of reconstituted drug**	**Melting point of powder (^oC)**	**Ceftriaxone**	**Sulbactam**	**Comment**
Control	1.045±0.054	6.9	202-207	93.5±0.5	Nil	Satisfactory
SB-A	1.575±0.078	6.5	196-198	90.9±0.9	86.8±1.6	Not satisfactory
SB-B	1.543±0.085	6.4	192-195	98.5±0.4	90.5±0.7	Satisfactory
SB-C	1.457±0.050	6.7	190-194	89.2±0.9	87.2±0.9	Not satisfactory
SB-D	1.498±0.065	6.4	200-201	87.6±1.2	89.6±0.9	Not satisfactory
SB-E	1.535±0.059	6.6	198-201	91.5±0.4	95.1±1.1	Satisfactory
SB-F	1.569±0.090	6.5	196-200	98.3±1.5	90.6±1.5	Satisfactory
SB-G	1.528±0.078	6.4	195-197	93.7±0.6	95.2±2.7	Satisfactory

Table IV: Bacterial endotoxin test for samples.

SAMPLE CODES	LIMULUS AMOEBOCYTE LYSATE TEST	
	Gel clot (EU/ml)	**Chromogenic (EU/ml)**
Control	0.06	0.06
SB-A	0.06	0.07
SB-B	0.06	0.06
SB-C	0.06	0.06
SB-D	0.08	0.08
SB-E	0.06	0.08
SB-F	0.07	0.08
SB-G	0.08	0.08

Table V: Antibacterial susceptibility testing for samples on bacterial typed strains and clinical isolates.

SAMPLE CODES	MINIMUM INHIBITORY CONCENTRATION (MIC) VALUES (MG/L)									
	Typed strains of bacteria					Bacterial clinical isolates				
	E. coli NCTC 10418	P. aeruginosa NCTC 10662	S. aureus NCTC 6571	S. pneumonia ATCC 49619	H. influenza ATCC 49247	Streptococcus spp.	Haemophilus spp	Neisseria spp	Pseudomonas spp	Staphylococcus spp
Control	0.07	0.09	1.25	0.02	0.08	0.05	0.03	0.03	0.08	0.09
SBA	0.01	0.02	0.08	0.01	0.03	0.01	0.02	0.01	0.05	0.04
SBB	0.02	0.02	0.04	0.01	0.02	0.01	0.03	0.01	0.04	0.03
SBC	0.03	0.04	0.07	0.03	0.05	0.01	0.05	0.02	0.04	0.05
SBD	0.02	0.05	0.06	0.03	0.02	0.03	0.04	0.03	0.05	0.03
SBE	0.02	0.01	0.08	0.02	0.03	0.01	0.03	0.01	0.04	0.03
SBF	0.01	0.02	0.05	0.02	0.02	0.02	0.02	0.01	0.03	0.02
SBG	0.02	0.01	0.06	0.01	0.02	0.01	0.02	0.02	0.02	0.03

NB: Average values are expressed for the MIC after triplicate determinations..

DISCUSSION

Majority (6/7; 83.3%) of the sampled products in this study emanated from India with only SB-F from China. Products from the Asian continent have been noted with pocket-friendly prices and many other pharmaceuticals in circulation in the study appear to be sourced from there. All products were however approved for marketing by National Agency for Food and Drug Administration and Control (NAFDAC). Physicochemical characteristics evaluated for the samples had values within the same range. The melting point of the powder revealed similar values for the products as they lay within the range 190-201 ^{o}C. The products considered here contain two molecules and the science of melting point determination for such mixture will influence the ultimate melting point. Melting point of mixtures usually appears with a broader range, often referred to as "pasty range". The observed melting point for the standard was higher (202-207^{o}C) than the sampled products. This was expected as the presence of another component with the ceftriaxone molecule characteristically produces lower values as seen in this result. Furthermore, the pH of solutions of reconstituted drug samples lay within the same values with a range (6.4 – 6.7). pH of injections should be restricted to a range that mimics the physiological values (pH 7 to 8). The pH of the standard in the literature is approximately 6.7.[11] The safety implications of the tested samples based on the observed values compare favourably with the standard.

None of the samples had powder adjudged to stick to vials in this study. Interaction of powder particles with water vapour at certain temperatures have been reported to cause flowing powders into cohesive or even sticky powders.[12] This attribute of non-stickiness compares similarly with the standard employed in this study. The uniformity of dosage units gave acceptable values for the mean ± SD values. The weights of powders were compared within each product and across the drug products. The deviation within each product revealed no significant difference in weight (P>0.05) and no statistical difference among the products (P>0.05). The products sampled were adjudged to be similar from the point of their physical assessment.

Samples SB-C and SB-D did not conform with the pharmacopoeia specification for content uniformity (12). Some variations observed for parameters in products quality, as it appears here, may have arisen from production processes rather than through deliberate falsification of drugs.[13] Excipient characteristics and efficient blending process have been reported to affect the final product quality of drugs.[13] Inappropriate packaging and certain storage conditions (extremes of humidity and temperature levels) may also be responsible for the shortfall in the observed values, from the official limits. A previous study on generic versions of ramipril tablets was found at an initial inspection of 17 brands with four out of 17 not meeting the official chemical content specification. On retest after 3 months a further four samples failing the chemical content test, due to the storage condition was observed.[14] Hydrolysis of active ingredients may occur leading to reduction in chemical content.

.

The biological test on the samples revealed acceptable quality characteristics as the vials examined for each sample were devoid of any microbial growth. Similarly, the pyrogen testing revealed acceptable outcome for the samples. The presence or absence of pyrogen in all aqueous parenteral is an official requirement for quality standards. The in vitro test was based on clotting reaction elicited in lysates of amoebocytes by small amount of endotoxin. All the samples passed the test. It is however noted that higher standards for pyrogen testing exists with higher sensitivities for both endotoxin and exogenous pyrogen for accurately detecting of pyrogenicity in humans.[15]

Minimum inhibitory concentration scores for the drug products. The limitation of these testing show that response in vivo may not always reflect the result of testing sensitivity of patient's pathogen in vitro. Some investigators have reported an excellent correlation between E-test results and broth dilution or agar dilution methods.[16] This was the reason E-test was included in the design of this study. The E-test also corroborated the outcome of the disk diffusion method

Antibiotic susceptibility testing methods performed in this article provided reliable results as procedures were followed as defined by the CLSI and manufacturers of the commercial products. However, there is considerable opportunity for improvement in the area of rapid and accurate recognition of bacterial resistance to antibiotics and responsible clinical judgment on choice of antibiotics for therapeutic success.

From observation of the inhibitory action of the standard compared with the test products on typed bacteria, there was extremely significant difference. The generic product had lower MIC against P. aeruginosa, S. aureus , H. influenza and for S. pneumonia. The superior activity of the combination with lactamase inhibitor is highlighted in this study. There is increasing report of resistance to ceftriaxone monotherapy in clinical settings against these organisms showcased.[17, 18] Similarly, the combination with lactamase inhibitor revealed higher inhibitory activity against the tested organisms. Noor and Munna (2015) reported that out of 248 bacterial isolates, which includes the ones employed in this research, 140 (56.5%) were found to be resistant to ceftriaxone. The decreased sensitivity of organisms to these ceftriaxone have been noted and reported worldwide.[19] As at 2007, drug resistant isolates of S. pneumonia have become increasingly common.[20,21] The importance of ceftriaxone enhanced antimicrobial activity is therefore, further emphasized.

CONCLUSION

This study highlights the physicochemical and microbiological indices of the sampled CTX-SBT products and the superiority/ importance of the co-presentation of the β-lactam molecule with β-lactamase inhibitor for the effective combat of organisms encountered in clinical settings.

REFERENCES

1. Venkatesh M, Bairavi VG, Sasikumar KC. Generic antibiotics industries: Challenges and implied strategies with regulatory perspectives. J Pharmacy and Bioallied Sci, 2011; 3(1): 101-108.
2. Barbour NP, Lipper RA. Introduction to Biopharmaceutics and its role in drug development. In Biopharmaceutics Application in Drug Development. Khrishna R Yu L (Eds).
3. Fisher JF, Merouch SO, Vobashery S. Bacterial Resistance to β-lactam Antibiotics Compelling opportunism, compelling opportunity: Chem Rev, 2005; 105 (2b: 395-424).
4. Munita JM. Arias CA. Mechanisms of antibiotic resistance. Microbiol spectrum, 2016; 4(2): 15-27.
5. Monaco M Giani T, Raffone M, Arena F, Giarcia-Fernandez A, Pollini S, Grundmann H, Pantosti A, Rossolini GM. Colistin Resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumonia*: a rapidly evolving problem in Italy. Eurosurveillance, 2014; 19-25.
6. Akova M, Sulbactam containing beta-lactam inhibitor combinations. Clin Microbiol Infect, 2008; 12(suppl 1): 185-199.
7. Sanchez ML, Jones RN. E test an antimicrobial susceptibility testing method with broad clinical and epidemiological application. Antimicrob Newsletter, 1992; 8: 1-7.
8. Shrivastata SN, Singh R, Tariq A, Sidiqui R, Yadav J, Negi PS, Chaudary M. A novel high performance liquid chromatographic method for simultaneous determination of ceftriaxone and sulbactam in sulbactomax. Int J Biomed Sci, 2009; 5(1): 37-43.
9. Lalitha MK. Manual on antimicrobial susceptibility testing: Indian Assoc Med Microbiol, 2004; 3-4.
10. CLSI. Performance Standards for Antimicrobial Susceptibility testing M02, M07 and M11, 29th Edition. January 2019; 1-24.
11. Hoffman-La Roche Inc. Product information on Ceftriaxone. Genentech Inc South San Francisco Ca 94080-4990, 2018.
12. British Pharmacopoeia. Her Majesty's Stationery Office, British Pharmacopoeia Commission, 2020.
13. Alyami H, Dahmesh E, Bowen J, Mohammed AR. An investigation into the effects of excipient particle size blending techniques and processing parameters on the homogeneity and content uniformity of a blend containing low-dose model drug. PLoS One, 2017; 12(6): e0178772. Doi;10.1371/journal.pone.0178772.
14. Angeli DG, Trezza C. quality and stability of ramipril generics/copies versus reference ramipril (tritace): a 3-month stability comparative study. Clin Drug Investig, 2009; 29: 667-676.
15. Eperon S, Groote DD, Werner-Felmayer G, Jungi TW. Human monocytoid cell lines as indicators of endotoxin. J Immunol Methods, 1996; 194; 121-129.

16. Rosser SJ, Alfa MJ, Hoban S, Kennedy J, Hardy GK. E-test versus agar dilution for antimicrobial susceptibility testing of viridians group streptococcus. J Clin Microbiol, 1999; 37(1): 26-30.
17. Levy S. The antibiotics Paradox: How misuse of antibiotics destroys their curative. Wers, Perceus, Cambridge, 2002.
18. Noor R, Munna MS. Emerging disease in Bangladesh. Curr Microbiol Res Perspective, 2015; 27(2): 49-53.
19. Geshe F, Mulisa E, Mekonnen M, Zeleks G, Antimicrobial resistance profile of different clinical isolates against third generation cephalosporins. J Pharmaceutics, 2018; Article ID: 5070742. https://doi.org/10.5070742.
20. Hawkins PA, Akpaka, Nurse-Lucas M, Gladstone R, Bentley SD, Breiman RF, McGee L, Swanston WH. Antimicrobial resistance determinants and susceptibility profiles of pneumococcal isolates recovered in Trinidad and Tobago. Jglob Antimicrob Resist, 2017; 11: 148-151.
21. Rice LB, Bonomo RA. Manual of Clinical Microbiology, American Society for Microbiology, 9th ed. Washington, DC, 2007; 1114–45.