

In Vitro Susceptibility Test of Different Clinical Isolates against Ceftriaxone

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Abstract:

Objectives: Because of the prevailing penicillin resistance in microorganisms, broad spectrum cephalosporins are used empirically specially in developing countries. The aim of this study is to determine the susceptibility pattern of different gram positive and gram negative pathogens against third generation cephalosporin-ceftriaxone to explore the existing effectiveness of this antibiotic.

Methods: 180 clinical isolates of different gram positive and gram negative pathogens including *P.mirabilis*, *S. typhi*, *P.aeruginosa*, *E. coli*, *S. aureus* and *Klebsiella* were collected from blood and urine samples of in-patients. 30 isolates of all species were tested against each of six brands of ceftriaxone using in vitro sensitivity tests by disc diffusion method (NCCLS criteria). The susceptibility limit was ≥ 21 mm zone of inhibition, while moderately susceptible was considered at 20-14 mm, and those isolates which showed >13 mm or no zone of inhibition were resistant to this antibacterial drug.

Results: Ceftriaxone was found most effective against *S. aureus*.

While 96.1% of the isolates showed susceptibility towards ceftriaxone, followed by *E. coli* (95%), *P. aeruginosa* (92.7%), *K. pneumonia* (89.4%) and *S. typhi* (87.2%). *P. mirabilis* showed lowest susceptibility amongst all the test organisms (83.8%).

Conclusion: Ceftriaxone can be used as a drug of choice in infections caused by *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and *S. typhi*. However, it should be used with other antimicrobial agents in order to increase its effectiveness against *P. mirabilis*.

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Introduction

Ceftriaxone is a broad spectrum third generation cephalosporin.¹ It is highly effective against Gram negative as well as Gram positive organisms. Ceftriaxone is unique because of its prolonged serum half-life, which permits once- or twice-daily dosing of this member of Cephalosporin family.² Ceftriaxone is stable to beta-lactamases, particularly those produced by Gram-negative bacteria. It has high potency against all the *Enterobacteriaceae*, *Haemophilus influenzae*, the *Nisseria* and most Gram-positive cocci except *Enterococcal spp.*³

Effectiveness of an antibiotic is compromised due to the emergence of antibiotic resistant microbes.⁴ Despite the constantly increasing need for new antimicrobial agents, antibiotic drug discovery and development seems to have greatly decelerated in recent years.⁵ Antimicrobial resistance has become a major health problem worldwide, affecting every country to some degree.² Encountered with the significant problem of advancing antimicrobial resistance, the global scientific community has attempted to find alternative solutions; one of the most promising ones is the evaluation and use of old antibiotic compounds.⁵

Due to the wide spread use of antibiotics in the health care

system the clinical isolates were developing resistance to different antibiotics. According to Technical Report by United States Agency for International Development, Ministry of Science and Technology, Government of Pakistan and Higher Education Commission, the tedious problem of antimicrobial resistance needs to develop resources to address the issue. This study aims to define contemporary susceptibility patterns of clinical isolates of gram positive and gram negative bacteria towards ceftriaxone. This study also gives an insight to the resistance pattern in different ceftriaxone sensitive bacteria and will help physicians, clinicians and consultants to decide its use in the most appropriate clinical infections.

Methods

180 Clinical isolates of each of six different bacterial species, namely *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhi* and *Pseudomonas aeruginosa* were collected from Jinnah Post Graduate Medical Centre (JPMC), National Institute of Child Health (NICH) and National Institute of Cardiovascular Diseases (NICVD) in Karachi from nosocomial infections. All the isolates were collected from the blood and urine samples of hospitalized patients.

Mueller-Hinton Agar was prepared by cooling the medium after autoclaving at the temperature of 121°C for 20 minutes. Susceptibility plates were prepared and stored at 2 - 8°C unless used. The broth culture was incubated at 37°C until it achieved the turbidity of the McFarland 0.5 standard.

Susceptibility plates were inoculated and allowed to dry for approximately 5 minutes, but no longer than 15 minutes, before adding the antibiotic disk. Six different brands of 1g ceftriaxone (injectable) available in the local market of Karachi were used to determine bacterial susceptibility. Antimicrobial susceptibility test was carried out using the disk diffusion technique. 30 µg Ceftriaxone discs were prepared by serial dilution.⁶ Ceftriaxone containing discs were applied to inoculated Muller-Hinton agar plates. After placement of ceftriaxone containing discs, the susceptibility plates were placed at 37°C in incubator for 16-18 hours. The procedure was repeated for all six brands of ceftriaxone. The breakpoints for susceptible, intermediate, resistant isolates were set as ≥21 mm (susceptible: S), 20-14 mm (intermediate

resistant: IR) and >13 mm or no zone of inhibition (resistant: R). After the incubation period, zone of inhibition appeared around the antibiotic discs. The diameter of the zone of inhibition including the diameter of the disk was measured, using sliding callipers held on the back of the inverted Petri plate. Sensitivity, intermediate resistance and resistances were determined by the zone of complete growth inhibition around each disk.

Results

The present study was designed to identify the susceptibility profile of different clinical isolates from nosocomial infections. The zones of inhibition around every disc were calculated in millimetre for all six types of bacteria against six different brands of ceftriaxone. The frequency of susceptible (with zone of inhibition ≥ to 21 mm), intermediate resistant (with zone of inhibition 20-14 mm) and resistant (with zone of inhibition ≤ 13 mm) are shown in Table 1. Susceptibility of these bacteria towards all of the six different brands of ceftriaxone is given cumulatively in Fig. 1.

Table 1: Susceptibility Pattern of Different Bacteria Towards Ceftriaxone

Ceftriaxone brand code	Cef-1			Cef-2			Cef-3			Cef-4			Cef-5			Cef-6		
	Frequency (n)																	
Name of bacteria	S	IR	R	S	IR	R	S	IR	R	S	IR	R	S	IR	R	S	IR	R
<i>Staphylococcus aureus</i>	30	-	-	30	-	-	28	-	2	27	-	3	28	-	2	30	-	-
<i>Escherichia coli</i>	30	-	-	30	-	-	29	-	1	29	-	1	27	-	3	26	-	4
<i>Pseudomonas aeruginosa</i>	27	-	3	30	-	-	30	-	-	26	1	3	27	1	2	27	-	3
<i>Klebsiella pneumonia</i>	27	-	3	25	2	3	26	-	4	26	2	2	27	-	3	27	-	3
<i>Salmonella typhi</i>	26	-	4	26	-	4	26	-	4	27	-	3	26	-	4	26	-	4
<i>Proteus mirabilis</i>	24	1	5	22	4	5	24	1	5	23	5	3	24	1	5	24	1	5

Cef 1-6: different brands of ceftriaxone, S= susceptible, IR=intermediate susceptible, R=resistance, n = 1080

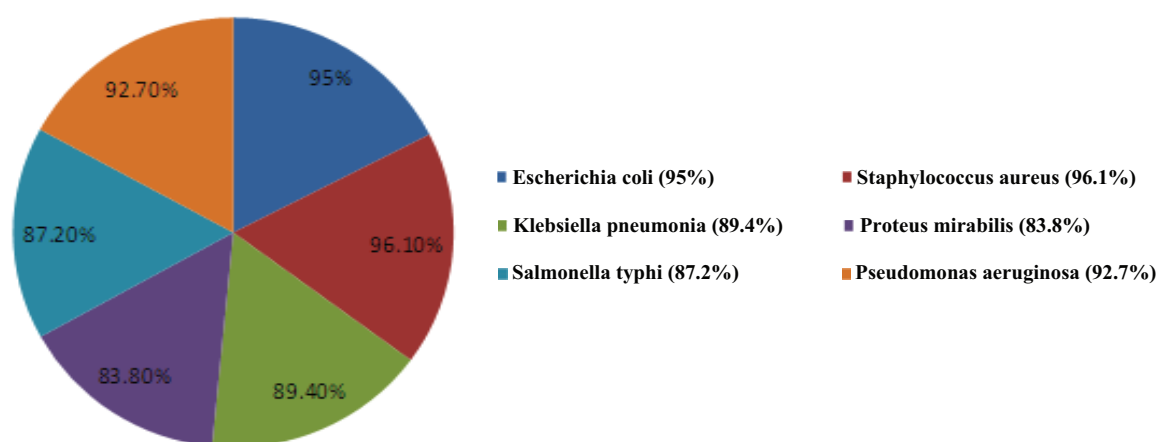


Figure 1: Susceptibility of different bacteria towards Ceftriaxone

Discussion

Microbial drug resistance is an inescapable consequence of the use of antimicrobial agents. The rate at which resistance occurs among microbial populations is often driven by the overuse and abuse of antimicrobial agents in many clinical settings.⁷ Differences in susceptibility to antibiotics by microorganisms has become a major factor in drug choice and success of treatment. Great concerns have been raised regarding emerging antimicrobial resistance among bacteria that may result in unpredictable antimicrobial susceptibility and failure of therapy.^{8,9}

Some trends in the antimicrobial resistance patterns and rates are universal, while others appear to be unique for specific regions. The Ceftriaxone, cephalosporin of the third generation is a potent antibiotic used in the treatment of life threatening infections. Since the rising trends of highly resistant organisms stresses the increasing importance of continuous surveillance system and stewardship of antibiotics.¹⁰ This study was conducted to determine the present sensitivity profile of different gram positive and gram negative bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhi* and *Pseudomonas aeruginosa*) in Karachi.

The use of ceftriaxone has increased for most infection causing organisms of gram positive and gram negative types as well as for aerobic and anaerobic organism.¹¹⁻²¹ This in vitro study of ceftriaxone shows that it is still effective against all these bacteria. All six different organisms showed susceptibility towards ceftriaxone. However, *Staphylococcus aureus* showed highest susceptibility against ceftriaxone. The result also favours another study conducted in Karachi in 2001, which found that ceftriaxone inhibited nearly 92% strains of *Staphylococcus aureus* and it is the most active β -lactam agent against this bacterium.²² *Escherichia coli* is a gram negative bacterium commonly harmless, but some can cause serious food poisoning in humans, and is occasionally responsible for costly product recalls and simulating blood and urinary infections.^{23,24} As gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against *Staphylococcus aureus* (a gram positive bacteria), and gram negatives such as *Klebsiella*, *Salmonella typhi* and *Pseudomonas aeruginosa*.²⁴

In this study, ceftriaxone proved to be highly active against *Escherichia coli* and it can be safely used in infections caused by *Escherichia coli*.

Over the past decade, the pathogenesis of infectious keratitis involving the opportunistic pathogen *Pseudomonas aeruginosa* has been investigated.²⁵ There has been rising trends of resistance noted in 2002 in *Pseudomonas aeruginosa* against third generation cephalosporin.²⁶ In the current study, 92.7% of the organisms

showed susceptibility towards third generation cephalosporin-ceftriaxone which highlights the significance of this antibacterial agent in infections caused by *Pseudomonas aeruginosa*.

A prospective study heralds that *E. coli* and *Klebsiella* are the most common organisms causing UTI in the metropolitan city of Karachi.²⁷ In this study, *Klebsiella* showed lesser sensitivity towards ceftriaxone compared to *Escherichia coli*, but still 89.4% of the isolates were susceptible. Only 10.6% of the *Klebsiella* isolates showed resistance as the antibiotic did not develop any zone of inhibition. *Klebsiella* was found to be more sensitive against meropenem and imipenem, amikacin, gentamicin and tobramycin, Cefipime and Aztreonam.²⁸ But the current findings prove that ceftriaxone is still effective against *Klebsiella* as 87.7% showed susceptibility. In a study conducted in 1992 in Karachi the *Salmonella typhi* responsible for typhoid fever were found sensitive to ceftriaxone.²² However, in the current study, *Salmonella typhi* showed 87.2% susceptibility (12.8% resistance) towards ceftriaxone, which means that ceftriaxone can still be used effectively against *S. typhi*. While *Proteus mirabilis*, a gram-negative, facultative anaerobic bacterium causing 90% of all 'Proteus' infections in humans. The study conducted in 2006, in Karachi, 70.4% of *Proteus mirabilis* were sensitive to ceftriaxone,²⁹ and this current study demonstrates that 78.3% of the *Proteus mirabilis* used in the invitro sensitivity test were susceptible to the ceftriaxone.

The present study proves that all six different brands of ceftriaxone are effective therapy for the treatment of infections caused by *Staphylococcus aureus*, *E. Coli*, *Ps. aeruginosa*, *K. pneumoniae*, *Salmonella typhi* and *Proteus mirabilis*. This report shall be helpful for physicians, prescribers and consultants to prescribe alternative antibiotic with cost effectiveness. There is need for appropriate national guidelines for the use of this antibacterial agent to avoid the development of resistance by sensitive organisms.

Conclusion

In reality, the extensive use of β -lactam antibiotics in hospitals and communities has created major evolutionary pressures in bacteria to evolve towards resistance. The β -lactamase antibiotics are the largest and currently most widely used antibacterial agents. Therefore, their resistance means a level of antimicrobial activity associated with a high likelihood of therapeutic failure.

The antibiotic policy guidelines preparation is the need of the hour to avoid any bitter experience of misuse of this class of drug therapy. The team efforts of the members comprising consultants, prescribing physicians, and members of health services team should participate in producing an effective plan

to make better decisions in this regards. In addition, surveillance programs should be conducted periodically to evaluate the sensitivity and susceptibility of these bacteria against prescribed antibiotics. Since the emergence of resistance remains silent for periods and takes time to become apparent, these antibiotics should be prescribed with cautions so that the bitter experience of antibiotic resistance can be avoided. These in vitro sensitivity studies also help in cost-effective prescribing of different brands of the antibiotic to the different patients with varying socioeconomic backgrounds. Overall, resistance patterns in different regions should be conducted so that the profiles of the specific region are maintained and monitored accordingly. This will therefore act as a guide towards the formulation of a comprehensive monitoring program of chemotherapy.

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References

- Nath SK, Foster GA, Mandell LA, Rotstein C. Antimicrobial activity of ceftriaxone versus cefotaxime: negative effect of serum albumin binding of ceftriaxone. *J Antimicrob Chemother* 1994 Jun;33(6):1239-1243.
- Masood H, Naqvi SB, Aslam N. Cost effective analysis of different brands of ceftriaxone available in Karachi Pakistan. *Pak J Pharmacol* 2008;25:13-19.
- Hwang KP, Tang YF, Shen YH. Activity of ertapenem, ciprofloxacin, ceftriaxone, piperacillin-tazobactam, and ampicillin-sulbactam against 12 common clinical isolates of community-acquired bacteremia. *J Microbiol Immunol Infect* 2009 Oct;42(5):433-438.
- Supp DM, Gardner J, Klingenberg JM, Neely AN. Antibiotic resistance in clinical isolates of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* does not impact sensitivity to human beta defensin 4. *Burns* 2009 Nov;35(7):949-955. Published online 6 Jun 2009.
- Liaqat I, Sumbal F, Sabri AN. Tetracycline and chloramphenicol efficiency against selected biofilm forming bacteria. *Curr Microbiol* 2009 Aug;59(2):212-220.
- Bell JM, Turnidge JD, Gales AC, Pfaller MA, Jones RN; Sentry APAC Study Group. Prevalence of extended spectrum β -lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998-99). *Diagn Microbiol Infect Dis* 2002 Mar;42(3):193-198.
- Khan S, Gupta DK, Khan DN. Comparative study of three antimicrobial drugs protocol (Ceftriaxone, Gentamicin/Amikacin and Metronidazole) versus two antimicrobial drugs protocol (Ceftriaxone and Metronidazole) in cases of intra-abdominal sepsis. *Kathmandu Univ Med J (KUMJ)* 2005 Jan-Mar;3(1):55-63.
- Huang TM, Lin TL, Wu CC. Antimicrobial susceptibility and resistance of chicken *Escherichia coli*, *Salmonella* spp., and *Pasteurella multocida* isolates. *Avian Dis* 2009 Mar;53(1):89-93.
- Khameneh ZR, Afshar AT. Antimicrobial susceptibility pattern of urinary tract pathogens. *Saudi J Kidney Dis Transpl* 2009 Mar;20(2):251-253.
- Irfan S, Idrees F, Mehraj V, Habib F, Adil S, Hasan R. Emergence of Carbapenem resistant Gram negative and vancomycin resistant Gram positive organisms in bacteremic isolates of febrile neutropenic patients: a descriptive study. *BMC Infect Dis* 2008;8:80.
- Zarakolu P, Sakizligil B, Unal S. [Antimicrobial resistance of *Neisseria gonorrhoeae* strains isolated from sex workers in Ankara]. *Mikrobiyol Bul* 2006 Jan-Apr;40(1-2):69-73.
- King A, Bethune L, Phillips I. In vitro activity of MDL 62,879 (GE2270 A) against aerobic gram-positive and anaerobic bacteria. *Antimicrob Agents Chemother* 1993 Apr;37(4):746-749.
- Madhulika U, Harish BN, Parija SC. Current pattern in antimicrobial susceptibility of *Salmonella* Typhi isolates in Pondicherry. *Indian J Med Res* 2004 Aug;120(2):111-114.
- Francioli P, Etienne J, Hoigné R, Thys JP, Gerber A. Treatment of streptococcal endocarditis with a single daily dose of ceftriaxone sodium for 4 weeks. Efficacy and outpatient treatment feasibility. *JAMA* 1992 Jan;267(2):264-267.
- Mamishi S, Pourakbari B, Ashtiani MH, Hashemi FB. Frequency of isolation and antimicrobial susceptibility of bacteria isolated from bloodstream infections at Children's Medical Center, Tehran, Iran, 1996-2000. *Int J Antimicrob Agents* 2005 Nov;26(5):373-379.
- Dunne EF, Fey PD, Kludt P, Reporter R, Mostashari F, Shillam P, et al. Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC β -lactamase. *JAMA* 2000 Dec;284(24):3151-3156.
- Hsueh PR, Chen WH, Luh KT. Relationships between antimicrobial use and antimicrobial resistance in Gram-negative bacteria causing nosocomial infections from 1991-2003 at a university hospital in Taiwan. *Int J Antimicrob Agents* 2005 Dec;26(6):463-472.
- Karlowsky A, Jones ME, Mayfield DC, Thornsberry C, Sahm DF. Ceftriaxone activity against Gram-positive and Gram-negative bacteria isolated in US clinical microbiology laboratories from 1996 to 2000: results from The Surveillance Network Database-USA. *International Journal of Antimicrobial Agents* 2002;19 (5): 2002, 413-426.
- Lee SC, Huang SS, Lee CW, Fung CP, Lee N, Shieh WB, et al. Comparative antimicrobial susceptibility of aerobic and facultative bacteria from community-acquired bacteremia to ertapenem in Taiwan. *BMC Infect Dis* 2007;7:79.
- Boyanova L, Kolarov R, Gergova G, Deliverska E, Madjarov J, Marinov M, et al. Anaerobic bacteria in 118 patients with deep-space head and neck infections from the University Hospital of Maxillofacial Surgery, Sofia, Bulgaria. *J Med Microbiol* 2006 Sep;55(9):1285-1289.
- George AK. Archiva SWI, Catherine, Monserrat, Carlyn C. Use of the National Committee for Clinical Laboratory Standards Guide Lies for Disk Diffusion Susceptibility Testing in New York State Laboratories. *J Clin Microbiol* 2000;38(9):3348.
- Shoab MH, Naqvi SB, Shiekh D, Hashmi HK. Cephalosporin Resistance And β -Lactamase Production In Clinical Isolates Of *Staphylococcus Aureus* In Karachi. *Pak J Pharm Sci* 2001;14(2):23-32.
- Vogt RL, Dippold L. *Escherichia coli* O157:H7 outbreak associated with consumption of ground beef, June-July 2002. *Public Health Rep* 2005 Mar-Apr;120(2):174-178.
- Abdul Jalil, 2Shafiq Ahmad Tariq, 2Iftikhar Ud Din Niazi. Comparative susceptibilities of *Escherichia coli* to ceftriaxone alone and in combination with clavulanic acid. *J. Med. Sci. (Peshawar, Print)* January 2009, Vol. 17, No. 1: 12-15.
- Fleiszig SM, Evans DJ. The pathogenesis of bacterial keratitis: studies with *Pseudomonas aeruginosa*. *Clin Exp Optom* 2002 Sep;85(5):271-278.
- Irfan S, Idrees F, Mehraj V, Habib F, Adil S, Hasan R. Emergence of Carbapenem resistant Gram negative and vancomycin resistant Gram positive organisms in bacteremic isolates of febrile neutropenic patients: a descriptive study. *BMC Infect Dis* 2008;8:80.
- Farooqi BJ, Shareeq F, Rizvi QK, Qureshi HS, Ashfaq MK. Changing pattern of antimicrobial susceptibility of organisms causing community acquired urinary tract infections. *J Pak Med Assoc* 2000 Nov;50(11):369-373.
- Naeem I, Naqvi BS, Hashmi K, Gauhar S. Paediatric nosocomial infections: resistance pattern of clinical isolates. *Pak J Pharm Sci* 2006 Jan;19(1):52-57.
- Iqbal SM, Udaipurwala IH, Shafiq AH, Mughal S. Chronic suppurative otitis media: disease pattern and drug sensitivity. *J Surg Pak* 2006;11(1):17-19.