REPORT

Sensitivity pattern of ceftriaxone against different clinical isolates

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Abstract: Emerging resistance against broad-spectrum antibiotics for standard empiric therapy is a global concern. Ceftriaxone (broad spectrum, third generation cephalosporin) is widely used in tertiary care settings to treat severe bacterial infections usually non-responsive to other antibiotics. The aim of the study is to evaluate the current sensitivity pattern of ceftriaxone (30µg/disk) among different clinical isolates. For this purpose, three hundred clinical isolates including *Escherichia coli* (25%), *Staphylococcus aureus* (30%), *Salmonella typhi* (17%) and *Klebsiella pneumoniae* (20%) were collected from different pathological laboratories of Karachi, Pakistan. The in-vitro sensitivity of different Gram positive and Gram-negative bacteria was determined by disk-diffusion technique using 0.5 McFarland standard. Results showed that ceftriaxone was highly sensitive against *Escherichia coli* (90%) and least sensitive against *Klebsiella pneumoniae* (65%). It is concluded that the sensitivity of ceftriaxone is progressively decreasing in comparison with past studies creating an alarming situation. Therefore, continuous surveillance is required to determine the current resistance status of clinical pathogens and for effective anti-microbial therapy.

Keywords: Ceftriaxone, resistance, antibiotic, clinical isolates, pathogens

INTRODUCTION

Microbial resistance against antibiotics is one of the growing problems of 21st century and now become a serious health concern globally. Primarily, pathogenic organisms were highly susceptible against existing antimicrobial agents; however due to acquisition of genetic mutation the effectiveness of broad-spectrum drugs has been decreased. As a consequence of genetic adaptations, the prevalence of multi-drug-resistant infections are also been increasing in clinical settings including, MRSA (methicillin resistant staphylococcus aureus), VISA (vancomycin-intermediate *S. aureus*), VRSA (vancomycin-resistant *S. aureus*), ESBL (Extended spectrum beta-lactamase), VRE (Vancomycin-resistant *Enterococcus*) and MRAB (Multidrug-resistant *A. baumannii*) (Appelbaum, 2007). The major factors for emergence of resistance are well documented involving inappropriate and misuse of antibiotics (Canton *et al*., 2013), selling of antibiotics without prescription, use of antibiotics in livestock (Mathew *et al*., 2007) leading to the creation of resistant strains. The lack of microbial susceptibility results in failure of therapy and financial burden on health care. Regular investigation and global attention is required to treat the emergent threat of antimicrobial resistance (Zehra *et al.,* 2010, Ali *et al.,* 2010). Surveillance studies have been carried out to identify the consumption as well as effectiveness of drugs in variety of treatments. This constant monitoring is required to establish the contemporary regimen and to treat different infections magnificently.

Ceftriaxone was introduced in 1985 as the first extended spectrum, semi synthetic, third generation intravenous cephalosporin in clinical practices (Lamb *et al.,* 2002). In contrast to the first and second-generation cephalosporin, ceftriaxone is more effective against Gram-negative bacteria. It is extensively used in treatment of skin infections (Gainer, 1991), soft tissues infections (Brown *et al.,* 1996) joint complains (Weiland *et al*., 2012) and other infections of enterobacteriaceae. However the production of extended spectrum beta lactamases (ESBL) by enterobacteriaceae has questioned the efficacy of the empiric use of ceftriaxone (Song *et al.,* 2009).

Ceftriaxone is also been utilizing in tertiary care units for management of gynecological infections and other sexually transmitted diseases like gonorrhea (Hustiq *et al.,* 2013). Effectiveness of the drug is also proved in pulmonary, blood and urine infections. Ceftriaxone is recommended as a first line drug for community acquired pneumonia (Lamb *et al.,* 2002), and as a substitute to other antibiotics such as fluoroquinolones for penicillin resistant isolates (Heffelfinger *et al.,* 2000, Bartlett *et al.,* 2000, Song *et al.,* 2009). It is also prescribed in acute otitis media in children as a first single dose regimen. This treatment is clinically considered as significant as amoxicillin, cefaclor, trimethoprim/ sulfamethoxazole, amoxicillin/clavaulate and high dose amoxicillin/ clavulanate. The pneumococcal resistance against different broad spectrum agents has increasing steadily;

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nevertheless most of the pneumococci are still susceptible to ceftriaxone (Lutter *et al.,* 2005, Rahman *et al.,* 2001).

This study is a surveillance study, conducted to determine the in-vitro susceptibility pattern of ceftriaxone against four common pathogens i.e. *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumonia* using disk diffusion method.

MATERIAL & METHOD

Collection of samples

A total of three hundred clinical isolates were collected from different pathological laboratories of Karachi (Essa's laboratories, Darul-Sehat hospital laboratories, Liaquat National hospital laboratories, Agha Khan Laboratories) from April 2012 to June 2013. These pathogens were isolated from blood, urine, stool, sputum and pus samples. Clinical microorganisms were identified on their colony characteristics in different media and through biochemical reactions. The isolates were inoculated in nutrient agar slants and then preserved at 4°C. Among Gram positive pathogens *Staphylococcus aureus* (n=90) was and from Gram negative the selected microorganisms were *Escherichia coli* (n=110), *Salmonella typhi* (n=40) and *Klebsiella pneumoniae* (n= 60).

Preparation of inoculum and susceptibility plates

Antimicrobial susceptibility of drug was determined using Kirby-Bauer method (Disk- Diffusion technique) according to CLSI (Clinical and Laboratory Standard Institute) guidelines (CLSI, 2012). Mueller Hinton Agar and broth were prepared as per instructions provided by manufacturer (Merck). All microbial culture tubes were incubated at 37ºC for 3-4 hours to develop the desired turbidity matched with 0.5 McFarland standard (Masood *et al.,* 2008). Antimicrobial disk of ceftriaxone 30µg/disk (Oxoid Ltd; (Basingstoke, Hampshire, England), was purchased from the commercial market. Microbial culture suspension was streaked on Muller Hinton medium plates using sterile, cotton swab and antimicrobial disks were then placed using sterile forceps.

Incubation of susceptibility plates

These plates were incubated in an incubator (Bunden Schutzart, B28-04-72367) at 37°C for 18-24 hours. All experimental work was done near a flame to avoid contamination.

Measurement of zones of inhibition

Zones of inhibition appeared around the disks were measured by placing the scale on the back of the petri dishes and compared to standards provided by CLSI (CLSI, 2012).

RESULTS

The pathogens were isolated from different sources as mentioned in table 1. The resistance of clinical isolates were determined by measuring zones of inhibition appeared around the disks. As per recommendations of CLSI 2012, pathogens are classified into three categories Resistant(R), intermediate Resistant (IR) and Sensitive (S). For such differentiation breakpoints are given in the manual of CLSI. Physicians are more concern to sensitive zones only rather intermediate ones to provide effective therapy. Clinically organisms having intermediate resistance profile, exert sub-therapeutic responses leading to treatment of failure.

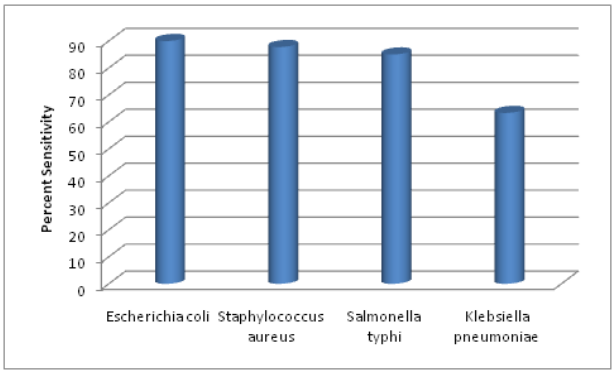

Fig. 1: Percentile sensitivity of ceftriaxone (30µg) against clinical isolates

The results of the present study showed that ceftriaxone (30µg), was highly susceptible against *E. coli* (90%). *S. aureus* was also sensitive to the drug but not to the extent of E. coli. However; *K. pneumoniae,* a gram negative facultative bacterium belongs to family Enterobacteriaceae was the least sensitive organism (65%). It is reported that *Klebsiella* organisms are becoming resistant to variety of antibiotic due to genetic adaptations. Management of community acquired infections caused by Klebsiella is becoming a threat to physicians owing to the development of extended-spectrum beta-lactamase (ESBL) *Klebsiella pneumoniae.*

DISCUSSION

Self-medication, irrational use of antibiotics, and incomplete course of therapy are the leading causes of resistance. Ceftriaxone is a third generation antibiotic, commonly used in treatments of urinary tract infections, throat and respiratory infections. Unfortunately the clinical pathogens have being started to acquire resistance against ceftriaxone, resulting in development of ceftriaxone resistant salmonella infections (Dunne *et al.,* 2000). However; such resistance against this extended spectrum cephalosporin is less endemic in developed countries in comparison to countries of poor economic status. The reported modes involve in acquisition of resistance is efflux pump operations and production of potent beta lactamases (Gutmann *et al.,* 1988, Bellido *et al.,* 1989). These genetic adaptations have created a fear full environment and application of successful therapy is now become challenge to physicians.

Table 1: Sources of clinical isolates

S. No.	Pathogens	Sources				
		Blood	Stool/urine	Skin pus	Sputum	Sample size
1	*Escherichia coli*	26	59	25	-	110
2	*Staphylococcus aureus*	29	23	38	-	90
3	*Salmonella typhi*	15	25	-	-	40
4	*Klebsiella pneumoniae*	18	13	12	17	60

Table 2: Resistance pattern of ceftriaxone against clinical isolates

S. No.	Pathogens	Resistant (R)	Intermediate Resistant (IR)	Sensitive (S)
1	*Escherichia coli*	06	05	99
2	*Staphylococcus aureus*	05	06	79
3	*Salmonella typhi*	03	03	34
5	*Klebsiella pneumoniae*	13	09	38

In this study, the current resistance status of ceftriaxone against common pathogens was determined. The rationale of being selecting E. coli, *S. aureus, S. typhi,* and *K. pneumonia* is that these pathogens are causative agents of majority infections. As a developing country, typhoid and pneumonia are highly endemic in population especially children are the most affected group of such infections. Ceftriaxone is one of the broad-spectrum antibiotics highly prescribed to treat variety of infections. Results showed that *E. coli* and *S. aureus* possessed excellent sensitivity against drug and their susceptibility pattern were almost in a similar fashion. *S. aureus,* is responsible for nosocomial infections especially respiratory, blood and skin diseases. The findings of study confirmed that ceftriaxone could be successfully use in the treatment of staphylococcal infections. Nevertheless, the retrospective data indicated the development of mild resistance of *S. aureus* from 2004 to 2007 (Tan *et al,* 2010).

In developing countries enteric fever is highly prevalent and accompanying with great health risks. The reported global mortality rate associated with enteric fever is 30% however; using appropriate regimen it could be reduced to 0.5% (Cooke and Wain, 2004). Different species of *Salmonella* are responsible for this enteric fever and recently resistant strains of *Salmonella* species is started appearing. In present surveillance study, *S. typhi* showed optimum antibacterial activity (85%) against ceftriaxone, reflecting the appropriateness of prescribing the drug against the infections of *S. typhi*. The clinical effectiveness of the drug against this intracellular pathogen has also been proved by past studies (Ekinci *et al.,* 2002; Masood and Aslam, 2010; Mothu *et al.,* 2011).

Ceftriaxone was widely used in the treatment of pneumonia and other types of chest infections. Owing to excessive and irrational use of newer beta lactam antibiotics *K. pneumonia* has developed resistance against cephalosporin (Gorbach 2001; Miriagou 2004). Ceftriaxone comprises of beta lactam ring but unfortunately organisms have become capable to produce lactamase enzyme responsible for the degradation of beta lactam ring. Strains of *klebsiella* and *E. coli* species were the first mentioned to have ESBLs (Lautenbach *et al.,* 2001). The findings of the present investigation showed that *K. Pneumonia* possessed highest resistance against ceftriaxone (37%) among other pathogens.

Above results present the overview that ceftriaxone is still sensitive against many strains of gram negative and gram positive bacteria. The gradual decrease in the susceptibility of 3rd generation cephalosporin reflects the alarming situation in future. Therefore it is needed to conduct monitoring programs for antibiotic rational, optimal and appropriate consumption to restrain the increasing trend of resistance.

CONCLUSION

Antibiotics are the most common therapeutic agents to cure different life threatening infections. The present study revealed that ceftriaxone is sensitive against different clinical pathogens, but it is evident from results that sufficient resistance against *K. Pneumoniae* has developed. All health care providers and hospital administrator should concurrently develop strategies and work efficiently to reduce the antimicrobial resistance, which would directly benefits patient safety. Additionally, national surveillance programs should be conducted routinely to maintain the efficacy of regimens and to avoid treatment failures.

REFERENCES

Ali SQ, Zehra A, Naqvi BS, Shah S and Bushra R (2010). Resistance pattern of ciprofloxacin against different pathogens. *OMJ.*, **25**(4): 294-298.

Appelbaum PC (2007). Reduced glycopeptide susceptibility in methicillin-resistant staphylococcus aureus (MRSA). *Int J Antimicrob Agents*, **30**(5): 398-408.

Ariza X, Castellote J, Lora-Tamayo J, Girbau A, Salord S, Rota R, Ariza J and Xiol X (2012). Risk factors for resistance to ceftriaxone and its impact on mortality in community, healthcare and nosocomial spontaneous bacterial peritonitis. *J. Hepatol.,* **56**(4): 825-832.

Bartlett JG, Dowell SF, Mandell LA, File TM, Musher DM and Fine MJ (2000). Practice guidelines for the management of community acquired pneumonia in adults. *Clin. Infect. Dis.,* **26**: 811-818.

Bellido F, Vladoianu IR, Auckenthaler R, Suter S, Wacker P, Then RL and Pechère JC (1989). Permeability and penicillin-binding protein alterations in *Salmonella muenchen*: stepwise resistance acquired during β-lactam therapy. *Antimicrob. Agents Chemother.,* **33**: 1113-1115.

Brown G, Chamberlain R, Goulding J,Clarke A (1996). Ceftriaxone versus cefazolin with probenecid for severe skin and soft tissue infections. *J. Emerg. Med.,* **14**(5): 547-551.

Cantón R, Horcajada JP, Oliver A, Garbajosa PR and Vila J (2013). Inappropriate use of antibiotics in hospitals: The complex relationship between antibiotic use and antimicrobial resistance. *Enferm. Infecc. Microbiol. Clin.*., **31**(Suppl 4): 3-11.

Cooke FJ and Wain J (2004). The emergence of antibiotic resistance in typhoid fever. *Travel Med. Infect. Dis.,* **2**: 67-74.

Duke T, Michael A, Mokela D, Wal T and Reeder J (2003). Chloramphenicol or ceftriaxone, or both, as treatment for meningitis in developing countries? *Arch.. Dis. Child.,* **88**: 536-539.

Dunne EF, Fey PD, Kludt P, Reporter R, Mostashari F, Shillam P, Wicklund J, Miller C, Holland B, Stamey K, Barrett TJ, Rasheed JK, Tenover FC, Ribot EM, Angulo FJ (2000). Emergence of domestically acquired ceftriaxone-resistant salmonella infections associated with AmpC beta-lactamase. *JAMA*, **284**(24): 3151-3156.

Ekinci B, Coban AY, Birinci A, Durupinar B and Erturk M (2002). *In vitro* effects of cefotaxime and ceftriaxone on Salmonella typhi within human monocyte-derived macrophages. *Clin. Microbiol. Infect.,* **8**(12): 810-813.

Gainer RB (1991). Ceftriaxone in treatment of serious infections. Skin and soft tissue infections. *Hosp. Pract.*, **26**(5): 24-30.

Gorbach SL (2001). Antimicrobial use in animal feed-time to stop. *N. Engl. J. Med.,* **345**: 1202-1203.

Gutmann L, Billot-Klein D, Williamson R, Goldstein FW, Mounier J, Acar JF and Collatz E (1988). Mutation of *Salmonella paratyphi*: A conferring cross-resistance to several groups of antibiotics by decreased permeability and loss of invasiveness. *Antimicrob. Agents Chemother.,* **32**(2):195-201.

Heffelfinger JD, Dowell SF, Jorgensen JH, Klugman KP, Mabry LR, Musher DM, Plouffe JF, Rakowsky A, Schuchat A and Whitney CG (2000). Management of community-acquired pneumonia in the era of pneumococcal resistance: A report from the drug-resistant streptococcus pneumoniae therapeutic working group. *Arch. Int. Med.,* **160**(10): 1399-408.

Hustig A, Bell C and Waddell R (2013). An audit of pharyngeal gonorrhoea treatment in a public sexual health clinic in Adelaide, South Australia. *Int. J. STD. AIDS*., **24**(5): 399-400.

Karlowsky JA, Jones ME, Mayfield DC, Thornsberry C and Sahm DF (2002). Ceftriaxone activity against gram-positive and gram-negative pathogens isolated in US clinical microbiology laboratories from 1996 to 2000: results from The Surveillance Network† (TSN†) Database-USA. *Int. J. Antimicrob. Agents,* **19**(5): 413-426.

Lamb HM, Ormrod D, Scott LJ and Figgitt DP (2002). Ceftriaxone: An update of its use in the management of community-acquired and nosocomial infections. *Drugs*, **62**(7): 1041-1089.

Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO (2001). Extended-spectrum β-Lactamase-producing *Escherichia coli* and *Klebsiella pneumonia* risk factors for infection and impact of resistance on outcomes. *Clin. Infect. Dis.,* **32**(8): 1162-1171.

Lewerenz J, Albrecht P, Tien ML, Henke N, Karumbayaram S, Kornblum HI, Wiedau-Pazos M, Schubert D, Maher P and Methner A (2009). Induction of Nrf2 and xCT are involved in the action of the neuroprotective antibiotic ceftriaxone *in vitro*. *J. Neurochem.,* **111**(2): 332-343.

Lutter SA, Currie ML, Mitz LB and Greenbaum LA (2005). Antibiotic resistance patterns in children hospitalized for urinary tract infections. *Arch. Pediatr. Adolesc. Med.*, **159**: 924-928.

Mathew AG, Cissell R and Liamthong S (2007). Antibiotic resistance in bacteria associated with food animals: A United States perspective of livestock production. *Food Borne. Pathog. Dis.,* **4**(2): 115-133.

Miriagou V (2004). Expanded-spectrum cephalosporin resistance in non-typhoid *Salmonella., Int. J. Antimicrob. Agents,* **23**: 547-555.

Muthu G, Suresh A, Sumathy G and Srivani R (2011). Studies on antimicrobial susceptibility pattern of Salmonella isolates from Chennai India. *IJPBS.,* **2**(2): 435-442.

NCCLS (2007). Performance standards for antimicrobial disk susceptibility tests; Approved standard, 17th ed., **27**(1): M100-S17.

Owens RC, Tessier P, Nightingale CH, Ambrose PG, Quintiliani R and Nicolau DP (2001). Pharmacodynamics of ceftriaxone and cefixime against community-acquired respiratory tract pathogens. *Int. J. Antimicrob. Agents* , **17**(6): 483-489.

Rahman M, Islam H, Ahmed D and Sack RB (2001). Emergence of multidrug resistant *Salmonella Gloucester* and *Salmonella typhimurium*in Bangladesh. *J. Health Popul. Nutr.,* **19**: 191-198.

Song KH, Jeon JH, Park WB, Park SW, Kim HB, Oh MD, Lee Kim NJ, Choe KW (2009). Clinical outcomes of spontaneous bacterial peritonitis due to extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella species: A retrospective matched case-control study. *BMC Infect. Dis.,* **9**: 41.

Swapan KN, Foster GA, Mandell LA and Rotstein C (1994). Antimicrobial activity of Ceftriaxone versus Cefotaxime: Negative effect of serum albumin binding of ceftriaxone. *J. Antimicrob. Chemother.*, **33**: 1239-1243.

Tan TY, Yong Ng LS, He J and Hsu Ly (2010). CTX-M and ampC β-lactamases contributing to increased prevalence of ceftriaxone-resistant *Escherichia coli* in changi general hospital, Singapore. *Diagn. Microbiol. Infect. Dis.,* **66**: 210-213.

Wieland BW, Marcantoni JR, Bommarito KM, Warren DK and Marschall J (2012). A retrospective comparison of ceftriaxone versus oxacillin for osteoarticular infections due to methicillin-susceptible *Staphylococcus aureus. Clin. Infect. Dis*., **54**(5): 585-590.

Zehra A, Naqvi BS, Bushra R and Ali SQ (2010). Comparative study on pattern of different pathogens against Cefixime and Cefepime. *JJPS.*, **3**(2): 145-156.