

3rd Generation Cephalosporin Resistance in *Klebsiella pneumoniae* from Pus Samples

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Abstract: Cephalosporins are considered to be the most important group of antibiotics today, which are safe, cheap and very effective for controlling bacterial infections caused by variety of bacteria including *Klebsiella pneumoniae*. The aim of this study was to check the effectiveness of 3rd generation cephalosporins against *Klebsiella pneumoniae* isolates from pus samples. Forty three isolates of *Klebsiella pneumoniae* were collected from pus samples and screened by Kirby-Bauer disk diffusion methods against 3rd generation cephalosporin antibiotics. All of the isolates showed varying level of resistance to tested cephalosporins viz ceftriaxone (74%), ceftazidime (70%), cefotaxime (81%) and ceftozoxime (63%). Since cephalosporins are widely used and considered very effective drugs in empirical therapy for controlling enterobacteriaceae infections, such an alarming resistance is a huge threat to human population. This overwhelming resistance is directly linked to the overuse of antibiotics. Prompt actions are needed to revise the policy of antibiotic therapy and regulate their prescriptions. Otherwise this continuous overuse will cause a complete failure of antibiotics treatment.

Key words: *Klebsiella pneumoniae* • Cephalosporins • MDR • ESBL

INTRODUCTION

Among the different classes of antibiotics commonly prescribed, cephalosporins are most widely used and their consumption reaches up to 50% of all the antibacterial agents prescribed [1]. Cephalosporins are the choice drugs for doctors and are most commonly prescribed because they are very safe for human health and can effectively target variety of bacteria [2]. Gram negative bacteria causing nosocomial infections of respiratory tract, gastrointestinal tract, urinary tract, blood and skin are the main target of cephalosporins [1]. In 1980s when use of cephalosporins antibiotics significantly increased, an overwhelming level of beta lactamase enzymes (Extended Spectrum Beta Lactamases) were reported from enterobacteriaceae causing both hospital and community infections [3].

Antibiotic resistance encountered in enterobacteriaceae against 3rd generation cephalosporins is because of Extended Spectrum Beta Lactamases (ESBL) [4] or AmpC

Beta lactamases [5]. ESBL enzymes produce resistance to beta lactam antibiotics such as penicillins, cephalosporins and aztreonam and are inhibited by beta lactamase inhibitors [6], while AmpC beta lactamases can even degrade cephamycins and are not inhibited by beta lactamase inhibitors [5]. Since the first report of ESBL producing bacteria from Germany [7], they have been found involved in many hospital outbreaks around the world [8]. ESBL-*Klebsiella pneumoniae* outbreaks have been reported from many countries including America [9] and Europe [10]. Cephalosporins resistant *Klebsiella pneumoniae* have been attributed to various epidemics of nosocomial infections and significant morbidity and mortality [11]. ESBL producing bacteria, including *Klebsiella pneumoniae*, are difficult to treat because the genes encoding ESBL enzymes often co-exist with other genes which confer resistance to many other important groups of antibiotics such as sulfonamides, aminoglycosides, chloramphenicol and tetracycline [12].

Consistent antibiotic surveillance to determine the resistance profiles of pathogenic bacteria is an important strategy for empirical therapy and identification of any emerging threats [13]. Various researchers have documented the resistance pattern of *Klebsiella pneumoniae* to 3rd generation cephalosporins [14], but consistent surveillance of resistance is necessary to check for any emerging threat [1]. The objective of this study was to check the resistance profile of *Klebsiella pneumoniae* isolates in pus samples against third generation cephalosporins antibiotics.

MATERIALS AND METHODS

***Klebsiella pneumoniae* Isolates:** Forty three isolates of *Klebsiella pneumoniae* isolated from pus samples of indoor and outdoor patients visited were collected from Pathology Lab. of Pakistan Railway General Hospital, Rawalpindi, were collected in multiple visits between March, 2012 and March 2013. These *Klebsiella pneumoniae* isolates were identified by the standard biochemical tests [15].

Cephalosporins Antibiotics Discs: Third generation cephalosporins antibiotics generally prescribed in hospitals were tested against *Klebsiella pneumoniae* isolates. Antibiotic discs and concentration of 3rd generation cephalosporins used in this study are, Ceftriaxone (30µg), Cefazidime (30µg), Cefotaxime (30µg) and Ceftozoxime (30µg).

Susceptibility Tests

Preparation of Inoculum: Each of the *Klebsiella pneumoniae* isolate was cultured in Mueller-Hinton Broth and culture was adjusted to 0.5 McFarland standard. Each of the isolate was then swabbed in sterile condition on Mueller-Hinton agar plates.

Disc Diffusion Tests: Antibiotics discs were placed on Mueller-Hinton agar plates and the plates were then incubated for 18 hours at 37 degree Celsius. Interpretation of resistance and susceptibility was done according to CLSI recommendations [16].

RESULTS

Klebsiella pneumoniae isolated from pus samples of indoor and outdoor patients were tested for susceptibility against third generation cephalosporins. All the *Klebsiella pneumoniae* isolates showed varying level of

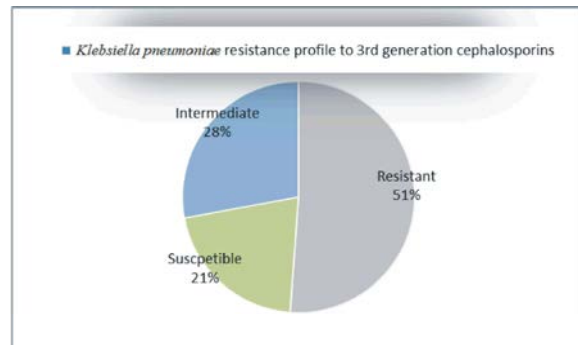


Fig. 1: Susceptibility profile of *Klebsiella pneumoniae* isolated pus samples

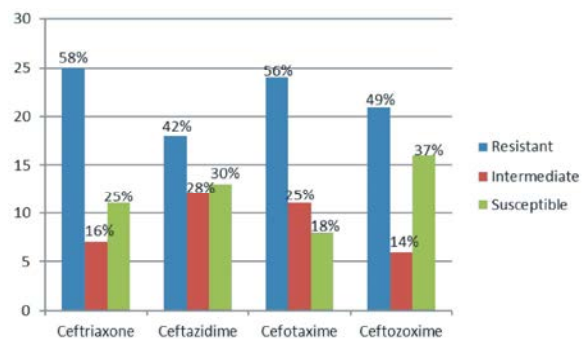


Fig. 2: *Klebsiella pneumoniae* susceptibility profile to 3rd generation cephalosporins

resistance to four types of tested third generation cephalosporins. 51% of all the isolates were found resistant, 28% were intermediate and only 21% were susceptible Fig.1. Four different types of 3rd generations cephalosporins were tested. *Klebsiella pneumoniae* isolates showed 74% resistance to ceftriaxone, 70% to ceftazidime, 81% to cefotaxime and 63% to ceftozoxime Fig.2.

DISCUSSIONS

Cephalosporins are very important group of antibiotics which are widely used in human medicine. Various generations of cephalosporins have been developed with, consequently, greater efficacy against the target bacteria. Third generation cephalosporins are largely used for treating infections of gram negative bacteria. *Klebsiella pneumoniae*, a gram negative opportunistic pathogen, is very important in this regard because it poses threatening level resistance to cephalosporins. Various authors around the world have reported varying level of resistance to 3rd generation cephalosporins. Our finding shows that 74% of the *Klebsiella pneumoniae* isolates were resistant to

ceftriaxone, 70% to ceftazidime, 81% to cefotaxime and 63% to ceftozoxime respectively. However, a much lower level of resistance was reported by Arsalan *et al.*, (2014) to ceftriaxone (14%) and ceftozoxime (35%) in *Klebsiella pneumoniae* isolates causing various types surgical site infections [17]. Similarly, in another study on *Klebsiella pneumoniae* isolates from surgical ward in 2010 [18], a considerable low level of resistance was reported to ceftazidime (33%), however cefotaxime was found to have 80% of the *Klebsiella pneumoniae* isolates resistant. In another study from Pakistan by Husain *et al.*, (2010) *Klebsiella pneumoniae* isolated from different infection sites were found to have 33% resistant to cefotaxime [19]. Jamil *et al.*, conducted a study in 2014, on *Klebsiella pneumoniae* isolates causing infections of urinary tract in children and found that 71% of the isolates were resistant to cefotaxime and about 72% to ceftazidime. In another report from a tertiary care hospital in Islamabad, Pakistan [20], *Klebsiella pneumoniae* isolates from various infections showed significantly high level of resistance to ceftriaxone (85%) and cefotaxime (82%) respectively [21]. This fluctuation in the resistance profile could be because of the different sites of infections, sample size and also depends on time interval for sampling. This varying level of resistance could also be attributed to prescription levels of particular cephalosporin type in different regions.

Cephalosporins are widely used since 1980 for treating gram negative pathogens but consistent level of resistance have been reported in bacteria by producing enzymes known as ESBL (Extended Spectrum Beta Lactamase) that degrade beta lactam antibiotics [22]. These ESBLs can efficiently degrade all 3rd generation cephalosporins but are inhibited by clavulanic acid. *Klebsiella pneumoniae* resistance to cephalosporins antibiotics is because of beta lactamases [23], of which TEM, SHV, CTX-M are very common. Strains harbouring the ESBL plasmid are posing greater threat because these strains not only pose resistance to cephalosporins, but can also spread resistance trait to other susceptible strains and make them resistant. That is one of the reasons that cephalosporins resistant *Klebsiella pneumoniae* have caused high level of morbidity and mortality in hospitalized patients [24]. Many such ESBL-*Klebsiella pneumoniae* outbreaks have been reported from Europe [10] and United States [24].

The prevalence of ESBL- *Klebsiella pneumoniae* varies in different regions. Falagas *et al.*, (2009) has reported 44% prevalence of ESBL *Klebsiella pneumoniae* in Latin America, 13.3% in Europe and 7.5%

in North America [25]. Among Asian countries, ESBL *Klebsiella pneumoniae* have been reported from Iran 77% [26], 51% China [27] and 58% India [28]. In Pakistan different studies show varying prevalence at different hospital set ups. A study conducted on *Klebsiella pneumoniae* isolated from hospitalized patients in Pakistan Institute of Medical Sciences [29], 35 out of 50 isolates (70%) were ESBL positive. Similarly, Afridi *et al.*, [30] has reported that 84.6% of *Klebsiella pneumoniae* isolates had Extended Spectrum Beta Lactamase enzymes, which are responsible for resistance to many beta lactam enzymes including third generation cephalosporins.

CONCLUSION

Infections caused by multi-drug resistant bacteria are a serious problem around the world. *Klebsiella pneumoniae*, an opportunistic pathogen, has often been found associated with this issue. Cephalosporin resistance in *Klebsiella pneumoniae* is a general measure of the rapid dissemination of ESBL positive isolates. ESBL-positive isolates rapidly spread in hospitalized patients, generally through the contaminated tools and hands of hospital staff. And if this continues greater uncontrolled morbidity will result with increased health care budgets. Along with limited treatment options available and poor management of diseases, drug resistant bacteria are posing the biggest threat to human health today. Continuous surveillance of antibiotic resistance in bacteria and consequently infections control measures should be promptly and strictly adapted.

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