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Susceptibility Profile of *Klebsiella pneumoniae* Isolates from Pus to β-Lactam/β-Lactamase Inhibitor Combinations

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Abstract: β-lactam antibiotics are the most important and frequently used antimicrobial agents worldwide. However, bacteria have circumvented their efficacy by producing enzymes-the β-lactamases that degrade β-lactam antibiotics. To overcome this resistance mechanism β-lactam/β-lactamase inhibitor combinations were developed. However resistance to such antibiotic formulation is also on the rise. *Klebsiella pneumoniae* clinical isolates from pus samples were screened for susceptibility on Kirby-Bauer disc diffusion methods against the three commercially available β-lactam/β-lactamase inhibitor combinations (amoxicillin/clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg), cefoperazone/sulbactam (75/30 µg). Amoxicillin-clavulanic acid showed significant resistance while piperacillin-tazobactam was found the most effective and exhibited maximum inhibition.

Key words: Antibiotics resistance · β-Lactam/β-Lactamase Inhibitors · *Klebsiella pneomoniae*

INTRODUCTION

Beta-lactam antibiotics are the most important and frequently prescribed medicines. The development and cheap availability of β- lactam antibiotics is one of the best contributions of medical sciences to society [1]. β-lactam antibiotics are frequently used in both developed and underdeveloped countries because they are very cheap and have negligible side effects on health. Because of their excessive use, variety of bacteria belonging to diverse families has become resistant. Resistant to β-lactam antibiotics in bacteria could be by three mechanisms; modification in the target site of antibiotic in bacteria, pushing the antibiotic out of the cell via efflux pumps, or by the production of β-lactamase enzymes which hydrolyse the β-lactam antibiotics [1]. Of the three types of resistance mechanisms exist, β-lactamase mediated resistance is posing a huge threat to health care system [2-4].

β-lactamases constitute a family of enzymes which can efficiently hydrolyse the β-lactam ring of the antibiotics [5-10]. The genes for β-lactamases could be present on chromosomes, or they may reside on mobile genetic elements like plasmid and transposons [5,11,12]. Since resistance is mounting in bacterial communities against the β-lactam antibiotics because either they replicate and give rise to a resistant generation or alternatively they share resistant genes with susceptible siblings and rendering them resistant. To overcome this bacterial strategy of resistance and keep the utility of β-lactam antibiotics, two ideas were undertaken either to design novel β-lactam antibiotics which the β-lactamases would not be capable to degrade, or design the β-lactam and β-lactamase inhibitor combinations in such a way that inhibiter inactivate the β-lactamase and β-lactam reaches its target in bacteria [13].

Of the five β-lactam/β-lactamase inhibitor formulations available; amoxicillin-clavulanic acid, ticarcillin-clavulanic, ampicillin-sulbactam and piperacillin-tazobactam are available in the United States, while cefoperazone-sulbactam is also used in several European countries, Japan and India [13].

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Amoxicillin-clavulanaic acid, cefoperazone-sulbactam, piperacillin-tazobactum are generally used in Pakistan. The combination of β-lactam with β-lactamase inhibitors seems a very effective strategy to combat the overwhelming resistance to this important class of drugs [14,15]. The inhibitors are structurally similar to penicillin, with a modified side chain which firmly bind and inhibit β-lactamases [2]. These inhibitors do not have any individual inhibition properties but when combined with β-lactam antibiotics they ensure the activity of β-lactam antibiotics [16-18]. These β-lactam-β-lactamase combinations have been found very effective against penicillinase, cephalosporinases and Extended Spectrum β-Lactamases [19]. β-lactam/β-lactamase inhibitors are capable to inhibit Class A β-lactamases, most frequently encountered of them are CTX-M, TEM and SHV, while generally they are susceptible to Class B, C and D β-lactamases [13,20-22].

The β-lactamase inhibitors generally prescribed in clinical settings in Pakistan are clavulanate, sulbactam, tazobactam combined with penicillin or cephalosporin derivatives. Clavulanic acid is a natural inhibitor first isolated from a bacteria in 1970 [23], while sulbactam and tazobactam are synthetic inhibitors. Sulbactam was developed in 1978 [24] and tazobactam was developed in [25]. As a usual practice in health care settings, clinicians start empirical therapy based on the most appropriate antibiotics depending on the infection and local pattern of resistance [2]. Continuous surveillance to determine the resistance pattern of bacteria in a community or hospital setting is very important. The aim of this study was to investigate the resistance trend in clinical isolates of *Klebsiella pneomoniae* against the three commercially available β-lactam-β-lactamase inhibitor combinations viz amoxicillin/ clavulanic acid, piperacillin/tazobactam and cefoperazone/sulbactam.

MATERIALS AND METHODS

Klebsiella Pneumoniae Isolates: A number of pus samples from indoor and outdoor patients in Pathology Lab, Railway General Hospital, Rawalpindi, Pakistan between March-September, 2012 were diagnosed to identify the infectious agents. Identification was done through standard biochemical tests [26]. 37 *klebsiella pneumoniae* isolates from pus samples were collected and screened for susceptibility against β-lactam/β-lactamase inhibitor combinations.

β-lactam/β-lactamase inhibitor combinations: Three β-lactam/β-lactamase inhibitor combinations which are commercially available and in clinical practice were tested for susceptibility against the clinical *Klebseilla pneumonia*e isolates from pus samples. Commercially available filter paper discs impregnated with the proper concentration of the three commercially available β-lactam antibiotic and β-lactamase inhibitor formulations viz; amoxicillin/ clavulanic acid (20/10 µg), piperacillin/ tazobactam (100/10 µg), cefoperazone/sulbactam (75/30 µg) were used.

Preparation of Inoculum: *Klebsiella pneumoniae* isolates were grown in Mueller-Hinton Broth and turbidity of the culture was adjusted to 0.5McFarland standards as per CLSI recommendations. These isolate were then swabbed under sterile conditions on Mueller-Hinton agar plates.

Disc diffusion tests and interpretation: Discs were placed on Mueller-Hinton agar plates and incubated overnight at 37 degree Celsius. Interpretation of resistance and susceptibility was done according to CLSI guidelines [27]. Isolates with intermediate level of resistance were included in the percentage of resistant isolates.

RESULTS

Thirty seven *Klebsiella pneumoniae* isolates were screened against the three commonly prescribed β-lactam/β-lactamase inhibitor combinations. Amoxicillin-clavulanic acid showed 57% resistance, 24% were resistant to cefoperazone-sulbactam and only 13% were found resistant to piperacillin-tazobactam.

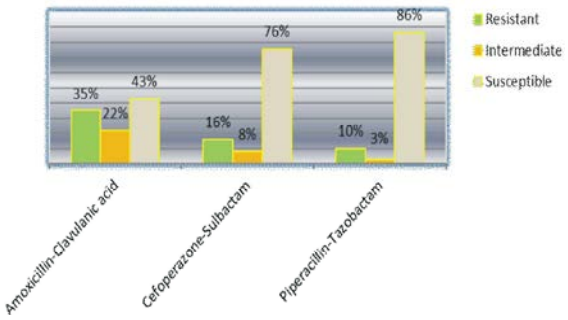

Fig. 1: Resistance profile of *Klebsiella pneumoniae* clinical isolates from pus sample to three commercially available β-lactam-β-lactamase inhibitor combinations

DISCUSSION

Our results confirm that amoxicillin/clavulanic acid has very poor activity against the *klebsiella pneumoniae* clinical isolates, more than half of the tested isolated (57%) were resistant to amoxicillin/clavulanic acid combinations. Our findings are in concordance with other reports from Pakistan. 50% resistance was reported to by Afridi and Farooqi [28], while 57% of *Klesbsiella pneumoniae* isolates were found resistant to amoxicillin-clavulanic acid [29].

Resistance to β-lactam/β-lactamase inhibitor combinations has been reported from worldwide. In a worldwide study from 138 hospitals (Africa, 3; Asia, 32; Europe, 44; Latin America, 19; Middle East, 3; North America, 30; and South Pacific, 7) *Klebsiella pneomoniae* isolates from various intra-abdominal infections showed 32% resistance to ampicillin-sulbactam and only 14% resistance to piperacillin-tazobactam [30].

Sarathbabu *et el.,* compared the resistance pattern of *Klebsiella pneumoniae* clinical isolates from pus, urine and sputum samples isolated in three consecutive years (2008, 2009, 2010) [31]. *Klebsiella pneumonia*e isolates showed almost same level of resistance to ampicillin-sulbactam from all sources *i.e.* pus (44%), sputum (46%) and urine (38%). [32] Reported comparatively low resistance (28.2%) to piparacillin-tazobactam. While a very negligible resistance (4%) was reported to amoxicillin-clavulanic acid from Nigeria [33], which is significantly low to the resistance (57%) we found [34] Reported that only 2% ESBL positive *Klebsiella pneumoniae* were resistant to cefoperazone-sulbactam while among ESBL negative only 9% were resistant. Dizbay *et al.,* Reported the co-resistance of β-lactam-β-lactamase inhibitor combination in carbapenem resistant *K. Pneumoniae*[35]. They found 100% resistant to amoxicillin-clavulanic acid in carbapenem resistant isolates while 75% were resistant in carbapenem susceptible isolates. Resistance level in carbapenem positive isolates to pipracillin-tazobactam was 81%, which is significantly high than carbapenem susceptible (35%). Similarly, co-resistance to cefoperazone-sulbactam in carbapenem resistant isolates were 62%, and only 32% were reported in susceptible.

Since bacteria become resistant because of the strong selection pressure imposed by antibiotics. Susceptible ones die but few get mutate and evolve which then replicate and give rise to a pool of resistant bacteria. These significant variations in the resistance level to different β-lactam/β-lactamase inhibitor combinations reported from worldwide could be because of the different exposure of bacteria to these antimicrobial agents. The reason for varying sensitivity to different β-lactamase inhibitor combinations could be that in different medical settings around the world one is more prescribed than the other.

Of the four classes (A, B, C, D) of β-lactamases, these inhibitors only inactivate the class A β-lactamases, of them TEM, SHV, CTX-M are very common. AmpC are class C β-lactamases which are also very common and can rapidly spread among bacterial communities but are resistant to β-lactamase inhibitors. AmpC β-lactamases also pose resistance to cephamycins (cefotetan, cefoxitin) [36-38]. There is an increasing trend in bacterial resistance to β-lactamases inhibitors and cephamycins in hospital infections and the reason could be the rapid dissemination of AmpC among these bacteria. However AmpC β-lactamases are susceptible to fourth generation cephalosporins (cefepime, cefpirom) and carbapenems [39, 40].

Additionally, the class D and B β-lactamases are also resistant to β-lactamase inhibitors. Classes D are not that common, while there are emerging threats among class B β-lactamses. NDM-1 is one such emerging threat. Class B, metallo β-lactamase, NDM-1 is resistant to various groups of antibiotics including the last resort antibiotics, cabapenem. NDM-1, was first reported from a Swedish patient in a hospital in New Delhi, India [41], but their prevalence has also been reported from Pakistan [42]. Metallo β-lactamases have been reported from various bacteria including *Pseudomonas spp, Acinetobacter spp* and members of Enterobacteriaceae [43,44].Resistance produced by bacteria to antimicrobials is an inevitable evolutionary process but the overuse, underuse, unnecessary and inappropriate use of these antimicrobial agents speed up this process. Antibiotic resistance spread by one of the two ways; clonal propagation of resistant bacteria among the communities and through unsanitary hospital practices by hospital staff, or alternatively, bacteria exchange resistant genetic material via plasmids, transposons, integrons and bacteriophages to other bacteria and make them resistant.

Antibiotic resistance is an alarming public health challenge worldwide. Multidrug resistant bacteria coupled with poor infection control practices in hospitals add to severity of the problem. Outbreaks in hospitals are becoming common, specially new born babies are at high risks because of their under developed immune system

[45]. Infections caused by resistant bacteria increase the treatment duration and costs hence put an extra economic burden on families and societies with poor health care budgets.

CONCLUSION

The biggest threat to human health today is antibiotic resistance in bacteria. Malpractices of antibiotic consumptions are challenging the blessings of modern medicine. Since antibiotics are the only most effective medicines to kill bacteria, overwhelming emergence of multi drug resistant bacteria have put the human race at constant risk. According to WHO [46], worldwide more than 50% of medicines are misprescribed or inappropriately sold on one hand, while on other hand 50% of patients do not take antibiotics correctly. WHO also identifies the main factors that accelerate the spread of resistance, including; Lack of diagnostic and standard therapeutic facilities, poor infection prevention and control practices and lack of a coordinated actions to control this huge threat. Prompt actions must be taken to combat the resistant bacteria otherwise human race will face a complete disaster.

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