CARBAPENEM RESISTANCE AND ANTIMICROBIAL OPTIONS IN *KLEBSIELLA PNEUMONIAE* ISOLATED FROM CLINICAL SPECIMENS OF ICU PATIENTS

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ABSTRACT

Objective: To determine frequency of carbapenem resistance and antimicrobial options among carbapenem resistant *Klebsiella pneumoniae* clinical isolates in ICU patients.

Material & Methods: This cross-sectional observational study was conducted at department of microbiology AFIP Rawalpindi in collaboration with ICU departments of CMH Rawalpindi, AFBMTC Rawalpindi, AFIU Rawalpindi and ALTU Rawalpindi from 1st Jan 2018 to 30st June 2018.

Isolates of *Klebsiella pneumoniae* yielded from various clinical specimens of ICU patients were identified as per standard protocols. Antimicrobial sensitivity testing was performed as per CLSI guidelines (EUCAST guidelines only for tigecycline), using Modified Kirby Bauer disc diffusion method and automated VITEK-2 systems version 08.01 for breakpoint MICs of colistin, tigecycline, imipenem and meropenem. Isolates resistant to imipenem, meropenem alone or both on MICs were considered carbapenem resistant.

Results: Among 130 *Klebsiella pneumoniae* clinical isolates, 83 (64 %) were identified as carbapenem resistant. The most common sources of clinical specimens includes; respiratory specimens 44 (34.0 %) followed by pus 35 (27.0 %), blood 24 (18.5 %), urine 15 (11.5 %), body fluids 06 (4.6 %), tissue 04 (3.1 %) and cerebrospinal fluid 02 (1.5 %). Colistin (4 %) and tigecycline (21 %) showed least resistance, while all other classes of antimicrobials were found highly resistant.

Conclusion: Carbapenem resistance among the clinical isolates of *Klebsiella pneumoniae* in ICU patients was to be found very high. It indicates that carbapenems cannot be used as empirical treatment option. Combination of colistin, tigecycline and meropenem is followed by culture directed de-escalation suggested to treat these superbugs in ICU settings.

Key Words: *Klebsiella pneumoniae,* Carbapenem resistance, Empirical therapy, ICU settings.

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INTRODUCTION

Klebsiella pneumoniae (KP) is a significant pathogen causing nosocomial infections and outbreaks with typically magnified problem of antibiotic resistance. Infections with multidrug-resistant (MDR) pathogens entail a significant increasing burden on both patients as well as healthcare system. Multi drug resistant (MDR) *Klebsiella pneumoniae* is one of those dangerous superbugs that are virtually becoming resistant against every antibiotic available these days [1].

Klebsiella pneumoniae have particular propensity for causing wide range of hospital acquired and community acquired infections ranging from urinary tract infections, skin and soft tissue infections, pneumonia to life threatening blood stream infections and meningitis [2]. However, it is the children, immunocompromised and patients admitted in intensive care units where the bug is in its most extreme and may cause fatalities. Other factors escalating its pathogenicity are its ability to spread rapidly, have intrinsic resistance and acquiring resistance to available therapeutic antimicrobial options [3].

For many years carbapenems were considered effective against such MDR isolates of *Klebsiella pneumoniae*. Now apparent developing resistance by *Klebsiella pneumoniae* against common and widely employed carbapenems also poses the most serious challenges for the medical fraternity. Various mechanisms contributing carbapenem resistance involve carbapenemases production, alterations in outer membrane permeability and efflux system up regulation [4].

Alarming spread (>50%) of carbapenem resistant *Klebsiella pneumoniae* (CRKP) globally is becoming common [5]. Most of times initial selection of treatment is empirical and is before identification of

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offending pathogen. Knowledge of details of regional epidemiology is essential for understanding specific state of affairs in developing antimicrobial resistance among common pathogens. Hence local epidemiological patterns should be evaluated actively rather than considering data published internationally. Aim of this study was to provide frequency of carbapenem resistance among *Klebsiella pneumoniae* (KP) clinical isolates as well as available antimicrobial treatment option in intensive care unit (ICU) patients of a tertiary care hospital.

MATERIAL AND METHODS

A Cross sectional observational study was conducted at department of microbiology AFIP Rawalpindi in collaboration with ICU departments of CMH Rawalpindi, AFBMTC Rawalpindi, AFIU Rawalpindi and ALTU Rawalpindi from 1st Jan to 30th June 2018.

All specimens were inoculated on blood agar and MacConkey's agar culture plates. Culture plates were incubated at 37°C in CO_2 incubator in ambient air for 24 to 48 hours. Identification of *Klebsiella pneumoniae* was done by colony morphology, Gram staining and biochemical reactions in API 20E (bioMerieux). All *Klebsiella pneumoniae* isolates yielded from different clinical specimens of patients admitted in ICU were included in study, while duplicate *Klebsiella pneumoniae* isolates having same antibiogram yielded from clinical specimens of same patient were excluded from the study.

A 0.5 McFarland equivalent suspension of isolated *Klebsiella pneumoniae* was inoculated on a Mueller Hinton (MH) agar plate. Antimicrobial sensitivity testing was performed using Modified Kirby Bauer disc diffusion method as per Clinical laboratory standard institute (CLSI) guidelines for amoxicillin-clavulanate 20/10µg, ceftriaxone 30µg, ceftazidime 30µg, cefepime 30µg, piperacillin-tazobactam 100/10µg, imipenem 10µg, meropenem 10µg, ciprofloxacin 5µg, gentamycin 10µg, amikacin 30µg, trimethoprim-sulfamethoxazole 1.25/23.75µg. For colistin and tigecycline susceptibility, breakpoint MICs were performed using automated VITEK-2 systems version 08.01. Results for colistin were recorded as per CLSI guidelines, while results of Tigecycline susceptibility were interpreted using EUCAST criteria for Enterobacteriaceae. All the isolates of *Klebsiella pneumoniae* with zone diameter less than 23mm against Imipenem or meropenem were further confirmed by break point MICs on automated Vitek-2 systems (version 2.01). Isolates resistant to either or both meropenem and imipenem were considered carbapenem resistant *Klebsiella pneumoniae* (CRKP) as per CLSI guidelines. *E.coli* ATCC 25922 was used as quality control strain. Data was analyzed using SPSS version 22. Mean and standard deviation was calculated for age. Descriptive statistics including frequencies and percentages were calculated for resistance of different antimicrobials against *Klebsiella pneumoniae* isolates.

RESULTS

A total of 130 samples received in laboratory for culture and sensitivity yielded growth of *Klebsiella pneumoniae*. Out of 130 samples 38 were collected from female and 92 from male patients. Mean age of patients ranged between 2 years to 93 years. Clinical specimens yielding the growth of *Klebsiella pneumoniae* were; respiratory specimens 44 (33.8 %), pus 35 (26.9 %), blood 24 (18.5 %), urine 15 (11.5 %), body fluids 06 (4.6 %), tissue 04 (3.1 %), cerebrospinal fluid 02 (1.5 %) as shown in figure-1.

Antimicrobial resistance pattern of *Klebsiella pneumoniae* isolates was recorded as amoxicillin-clavulanate 113 (87 %), ceftriaxone 107 (82 %), ceftazidime 107 (82 %), ciprofloxacin 104 (80 %), tazobactam/ piperacillin 103 (79 %), cefepime 102 (78 %), gentamycin 84 (65 %), trimethoprim / sulfamethoxazole 83 (64 %), meropenem 83 (64 %), amikacin 74 (57 %), imipenem 77 (59 %), tigecycline 27 (21 %), colistin 4 (3 %) as shown in figure 2. Out of 130 isolates of *Klebsiella pneumoniae* 83 (64 %) were identified as carbapenem resistant *Klebsiella pneumoniae* (CRKP). Antimicrobial resistance pattern of CRKP is shown in table-1. Among CRKP isolates 77 were resistant to both meropenem and imipenem. However, remaining 6 isolates were resistant to only meropenem. Two isolates were found pan drug resistant; one was isolated from blood culture specimen and other from respiratory specimen.

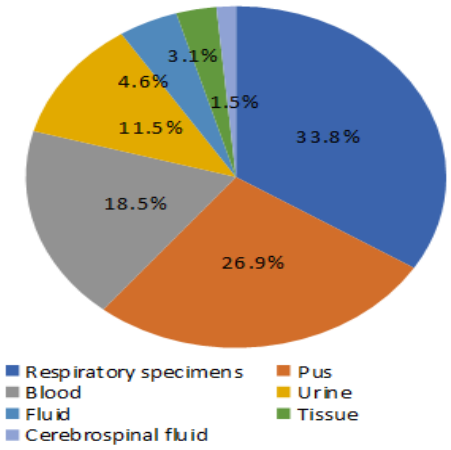

Figure-1: Specimen wise break down of *klebsiella pneumoniae* isolates (n=130).

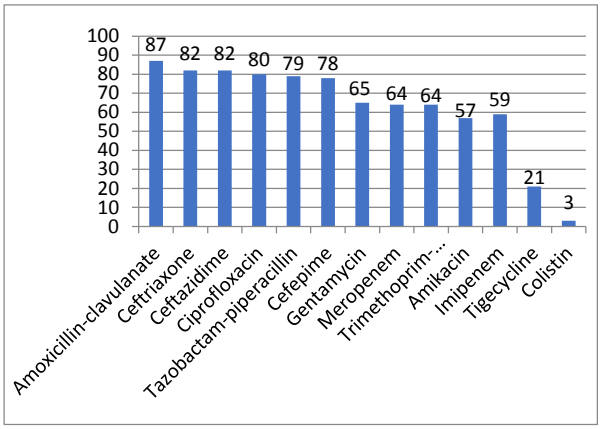

Figure-2: Resistance pattern of *Klebsiella pneumoniae* clinical isolates in ICU patients (n=130)

Table-1: Antibiogram of carbapenem resistant *Klebsiella pneumoniae* (CRKP) clinical isolates (n=83)

Antimicrobials tested	Resistance frequencies (%)
Amoxicillin-clavulanate	83 (100)
Cefepime	80 (96.3)
Ceftriaxone	82 (98.7)
Ceftazidime	82 (98.7)
Tazobactam-piperacillin	79 (95.1)
Ciprofloxacin	80 (96.3)
Trimethoprim-sulfamethoxazole	61 (73.4)
Gentamycin	69 (83.1)
Amikacin	73 (87.9)
Tigecycline	27 (32.5)
Colistin	4 (4.8)

DISCUSSION

Emergence of MDR and XDR superbugs has put progressive burden on health care systems as well as increased the morbidity and mortality due to limited treatments options for treating such infections. In our study out of 130 *Klebsiella pneumoniae* clinical isolates from ICU, 64 % were carbapenem resistant. Whereas in case of other antimicrobials, resistance was almost greater than 70 % in ascending order from trimethoprim - sulfamethoxazole, aminoglycosides, ciprofloxacin, β lactam-β lactamase inhibitor combinations to cephalosporins. Similar resistance has been observed from different regions of world [6]. A study conducted in Henan general hospital from china observed greater than 57 % resistance to carbapenems in ICU patients [7]. A descriptive study from Colombia comprising of 3 years, provided evidence from 23 hospitals for rising trends of resistance (> 60 %) to carbapenems [8]. Another study conducted in Spain emphasized on carbapenem resistance (87.7 %) in most frequently isolated pathogen K*lebsiella pneumoniae* from urinary samples [9].

These results are striking as cephalosporins, carbapenems and aminoglycosides are widely used as empirical therapy in most hospital settings. From our local antibiogram, it was also observed that all isolates that were resistant to imipenem were also resistant to meropenem. However, some of the isolates were found to be sensitive to imipenem but were resistant to meropenem indicating meropenem resistance greater than imipenem. Likewise, increased amikacin resistance was observed as compared to gentamycin. This can be most likely due to increased use of meropenem and amikacin as compared to imipenem and gentamicin in hospital settings.

Tigecycline and colistin showed lower level of resistance i.e. 32.5 % and 4.8 % respectively as compared to other antimicrobials tested. Nonetheless, 4.8 % resistance to colistin is also alarming as this is considered as the last resort treatment. In our study two isolates were pan-drug resistant; one was yielded from blood culture and other from respiratory specimen. Both patients died despite putting all available possible efforts. Pan-drug resistance among CRKP isolates was also reported from 3 hospitals of United Arab Emirates having same genotypes [10]. In 2016 a case of pan-drug resistant *klebsiella pneumoniae* was reported in Nevada also, leading to death of patient despite putting all possible efforts to treat with 26 available antimicrobials [11]. Moreover, there are studies that show developing resistance against colistin during treatment of CRKP [10-12].

The results of our study draw attention to the call for supporting WHO recommendations in discovery, research and development of newer antimicrobials for CRKP. However, till the advent of newer antimicrobials, combinations of different antimicrobial for synergistic activity can be used. There are studies that showed synergistic effect of colistin and meropenem. Adding colistin seems to increase the antibacterial activity of meropenem even if the isolate is resistant to colistin [13]. According to one of case report, a kidney transplant patient having infection with MDR *Klebsiella pneumoniae* was successfully treated with combination of carbapenems despite the elevated MICs [14]. Another patient having infection with pan drug resistant KP was successfully treated with combination of high dose tigecycline and colistin [15]. However, this combination may cause life threatening side effects if therapy is not monitored carefully.

In future clinicians may become dependent on second line agents colistin/ polymyxin B and

tigecycline. Further research is required to explore the zones for tigecycline as CLSI and EUCAST have not given the zones of inhibition for tigecycline.

CONCLUSION

Carbapenem resistance among the clinical isolates of *Klebsiella pneumoniae* in ICU patients was very high. It indicates carbapenems alone cannot be used as empirical treatment option as KP is most commonly isolated bug in ICU settings. All isolates were found resistant to almost all classes of antimicrobials except colistin and tigecycline. Combination of colistin, tigecycline and meropenem for empirical therapy, as in other studies, followed by culture directed de-escalation is suggested to treat these superbugs in ICU settings. Appearance of PDR strains in our ICUs is alarming situation as their spread can result not only high morbidity, mortality but also high financial burden. Observing hospital infection control policies and antimicrobial stewardship programs with true spirit is need of time to curtail antimicrobial resistance and spread of these super bugs.

AUTHORS CONTRIBUTION

Wajid Hussain: Principal author, data collection, paper writing.
Fatima Tuz Zahra: Data analysis, results compilation.
Shumaila Farman: Literature review, paper writing
Nadia Tayyab: Literature review, proof reading
Gohar Zaman: Data analysis, project approval
Muhammad Tahir Khadim: Supervision of project

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