Current efficacy of antibiotics against Klebsiella isolates from urine samples – A multi-centric experience in Karachi

Farhan Essa Abdullah[1], Ammara Mushtaq[1]*, Mubashira Irshad[1], Hiba Rauf[1], Noureen Afzal[2] and Abdur Rasheed[1]

[1]Dow University of Health Sciences, Karachi, Pakistan
[2]Department of Pediatrics, Aga Khan University Hospital, Karachi

Abstract: Due to emergence of bacterial resistant strains, the effectiveness of current antibiotic treatment without culture/sensitivity testing is questionable. Our study aims to assess the present sensitivity profiles of Klebsiella isolates from urine samples and provide options for empiric prescription in critically ill patients. *Klebsiella pneumoniae* isolates collected over a period of 28 months till January 2011 from 1,617 urine samples of subjects presenting with Urinary Tract Infections were identified at a local diagnostic lab using standard protocol and subjected to Kirby-Bauer disk diffusion sensitivity testing. MICs were also estimated by E-nephelometry. Among 20 drugs used, low sensitivity was found to amoxicillin (0.1%), doxycycline (11.5%), nitrofurantoin (15.5%), amoxiclav (18.2%), gentamicin (35.4%), pipemidic acid, cephradine (40.3%) and cotrimoxazole (43.1%). The isolates were more sensitive to cefuroxime (55.9%), cefixime (57.7%), ciprofloxacin (62.5%), ofloxacin (63%), ceftriaxone (66.2%), ceftazidime (66.4%), cefotaxime (66.6%), fosfomycin (77.5%) and amikacin (89.4). Most effective were cefroperazone.sulbactam (95.8%), piperacillin.tazobactam (95.7%) and imipenem (97.7%). Self-medication, lack of awareness, and the misuse of antibiotics by doctors has exacerbated the menace of microbial resistance. The study warrants the prudent choice of drugs in adherence with prevailing sensitivity profiles.

Keywords: *Klebsiella pneumoniae*, antibiotics, antimicrobial resistance, clinical isolates, urinary tract infection.

INTRODUCTION

Klebsiella pneumoniae carbapenemase (KPC) - producing isolates have been reported in various countries including USA, France, Sweden, Norway, Brazil, Poland, China, Israel and Greece (Hirsch and Tam, 2010). Klebsiella is said to be responsible for 10% of nosocomial bacterial infections (Amin *et al*., 2009). Despite large burden of infections in developing countries, there is serious lack of epidemiological data addressing risk factors, incidence and management (Ganatra and Zaidi, 2010). Increase in its resistance is attributed to overuse of antibiotics and the rapid evolution of complex resistance mechanisms in bacteria (Gootz, 2010). Patients with prior antibiotic exposure exhibit more resistance and are associated with increased mortality (Johnson *et al*., 2011).

Multidrug resistance in gram negative bacteria is apparently increasing in association with plasmid- and chromosomal-encoded- beta-lactamases (Bush, 2010). Data reported to the CDC showed that carbapenem-resistant *K. pneumoniae* (CPKP) of all Klebsiella isolates increased from <1% in 2000 to 8% in 2007 and their treatment had not been established (Hirsch and Tam, 2010). Klebsiella has reportedly been the second most commonly encountered septic organism in patients (Javed and Memon, 2009; Yoon *et al*., 2011; Johnson *et al*., 2011), the most prevalent organism in outdoor patients and in patients with Urinary Tract Infections (UTI) (Mumtaz *et al*., 2007; Farooqi *et al.*, 2000), and the second most common uropathogen involved in nosocomial infection (Khan *et al.,* 2010).

With emergence of highly resistant KPC- producing bacteria, the available choice of treatment is becoming narrower. Carbapenems like imipenem and meropenem are often the last resort for patients with Klebsiella infections like pneumonia or UTI. Alarmingly, there have been cases of CRKP infections with no other antibiotics currently in market to treat such resistant cases, thereby leading to enhanced focus on prevention of such infections in hospital and community settings. Though antibiotic resistance is a global issue, resistant organisms may be more prevalent in developing countries, including Pakistan, than in the Western world (Lagamayo, 2008). A possible reason for this could be that in a country like Pakistan, microbiological diagnosis is not available to 80% of the population, leaving doctors with no choice but to prescribe multiple antibiotics (Mushtaq *et al*., 2012). A study in Pakistan accordingly witnessed an increase in antibiotic resistance, for example, among *K. pneumoniae* causing UTI (Ullah *et al.,* 2009).

Previous studies done in Pakistan on susceptibility patterns of pathogens causing UTI, tested only a few antibiotics, ignoring other pertinent antibiotic classes (Khan and Ahmed, 2001) and hence left clinicians with hardly any options for adequate prescription. Also, these

**Corresponding author:* e-mail: ammara.mushtaq@live.com

studies were single-centered and consequently not representative of city population as a whole (Farooqui *et al*., 2000; Khan and Ahmed, 2001). However, since resistance patterns continue to evolve, it is imperative to ascertain resistance patterns periodically to gauge the extent of the occurrence and provide clinicians with the provision to improve outcomes of experiential treatment. We therefore aim to determine the current resistance profile of *K. pneumoniae* isolated from patient urine samples collected in a diagnostic lab and its branches in Karachi as a means to offer wider drug choice for empirical therapy.

MATERIALS AND METHODS

Design and setting

Our review was a multi-centric retrospective study done from November 2008 to January 2011. Samples were collected from routine patients coming to all 19 branches of Dr. Essa's Laboratory and Diagnostic Centre in key areas of Karachi, Pakistan.

Clinical isolates

A total of 1,617 mid-stream clean catch urine samples yielding *K. pneumoniae* were included in the study. Isolates were identified by standard protocol and API-20 E system (Biomerieux) and subjected to Kirby-Bauer disk diffusion sensitivity. MICs were also estimated by E-nephlometry. Clinical isolates were tested for their sensitivity; resistance or intermediate response to these 20 locally available drugs using Oxoid discs: amikacin, gentamicin, amoxicillin, amoxiclav (Augmentin), imipenem, piperacillin.tazobactum, cephradine, cefuroxime, cefixime, cefotaxime, ceftazidime, ceftriaxone, fosfomycin, pipemidic acid, oflaxacin, ciprofloxacin, doxycycline, cotrimoxazole, nitrofurantoin and cefoperazone-sulbactam. Zone diameters for each antibiotic were interpreted as Resistant, Intermediate or Sensitive. The antibiotics were from Oxoid. The zone diameters were estimated using calipers and according to the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1987) and the WHO.

Patient data

Subjects were either self-referrals or referred by physicians. Data of age, sex, date of investigation and any history of antibiotic usage were recorded from patients presenting with suggestive UTI. Data was analyzed using SPSS version 16.0. In case of missing values in records, the reported percentage in results is the valid percentage, ignoring missing values.

RESULTS

Out of a total of 1,617 isolates of *K. pneumoniae* reviewed, 19.3% (*n*=312) were from males and 80.7% (*n*=1302) from females. Table 1 shows sensitivity profile of all 20 antibiotics used: A total of 22 isolates were carbapenem-resistant. The resistance profile of imipenem is shown in fig. 1. Popularly prescribed drugs that performed poorly included amoxiclav (Augmentin) (18.2%), nitrofurantoin (15.5%), doxycycline (11.5%),

Table 1: Sensitivity profile of antibiotics against *Klebsiella pneumoniae*

Name of Antibiotics	Sensitivity Percentages	Resistance Percentages	Intermediate Percentages
Imipenem	97.7	1.4	0.9
Piperacillin.Tazobactam	95.7	2.1	2.1
Cefoperazone.Sulbactam	95.8	1.4	2.8
Amikacin	89.4	4.4	6.3
Fosfomycin	77.5	10.7	11.9
Cefotaxime	66.6	26.3	7.1
Ceftazidime	66.4	26.7	6.9
Ceftriaxone	66.2	26.6	7.2
Ofloxacin	63	33.4	3.7
Ciprofloxacin	62.5	34.2	3.3
Cefixime	57.7	36.1	6.2
Cefuroxime	55.9	37.0	7.0
Cotrimoxazole	43.1	53.8	3.2
Cephradine	40.3	48.9	10.8
Pipemidic acid	36.6	54.6	8.8
Gentamicin	35.4	46.7	17.8
Augmentin (amoxiclav)	18.2	76.7	5.1
Nitrofurantoin	15.5	30.5	54.1
Doxycycline	11.5	81.2	7.3
Amoxicillin	0.1	99.6	0.3

and particularly amoxicillin (0.1%).

A majority of the subjects (31.7%; *n*=497) were aged 15-30 years. The mean age of our patients was 38.91 ± 21.53 with a range of 0-94. Fig. 2 shows age-wise distribution of the cases.

DISCUSSION

Clinically, UTI is said to exist when 10^5 bacteria or more per ml of urine are found in a mid-stream specimen (Khan *et al*., 2010), a common baseline which was adhered to in our retrospective data. *K. pneumoniae*, an important uropathogen, is also said to be the most common cause of illness in humans after respiratory tract infections (Ram *et al*., 2000), thereby demanding tailored treatment.

In our study, imipenem with an efficacy of 97.7% was found to be the most effective drug against *K. pneumoniae* isolates as has been reported by Shah *et al*. (2010) and Mehrgan *et al*. (2010). Our results are also consistent with those observed recently on the efficacy of the drug (Akhtar, 2010; Hawser *et al*., 2011) and was more than the 92.5% sensitivity to carbapenems reported in Islamabad (Amin *et al*., 2009) and 85.1% in Egypt (Ashour and El-Sharif *et al*., 2009). One study in India showed only 46.66% antibiotic efficacy for imipenem (Shah et al., 2010), while another study also in India

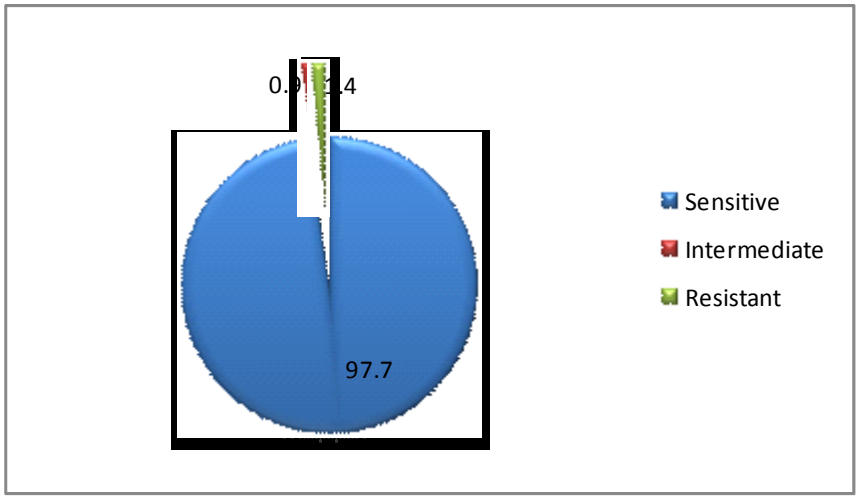

Fig. 1: Carbapenem (Imipenem) resistance profile.

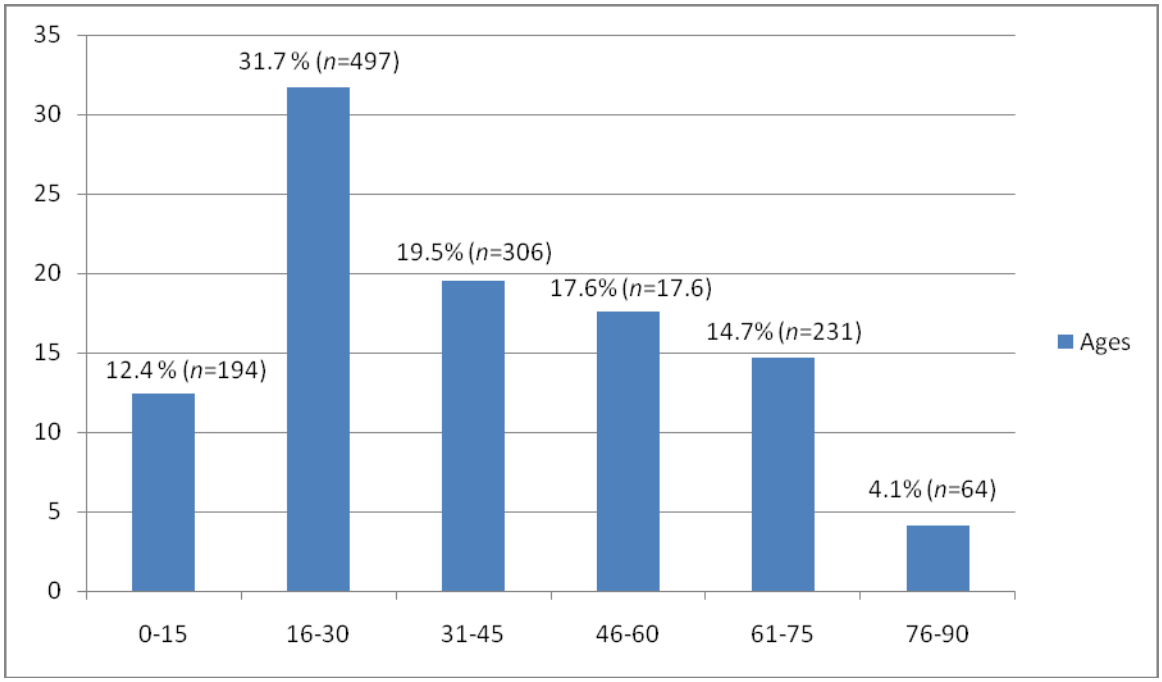

Fig. 2: Percentages of Age-wise distribution of cases.

considered imipenem efficacy to be as high as 99.2% (Abhilash *et al*., 2010) although Klebsiella isolates have been showing increased resistances the world over (Ullah *et al.,* 2009).

Amoxicillin had efficacy of 0.1%, which was consistent with findings in other studies such as that of Ullah *et al*. (2009). Incidentally, our data revealed that only one patient was sensitive to this antibiotic.

In addition, the first generation cephalosporin, cephradine, showed low (40.3%) sensitivity in our study, the second generation, cefuroxime, showed 55.9% efficacy, while the third generation cephalosporins; cefixime, ceftriaxone, ceftazidime and cefotaxime showed 57.7%, 66.2%, 66.4 and 66.6% efficacy respectively. In Egypt, cefotaxime, which along with ceftazidime is a potential marker for ESBL production, was reported to be significantly less effective than in our study (Ashour and El-Sharif, 2009). The same study reported ceftazidime sensitivity of 52% compared with our 66.4%, ceftriaxone sensitivity of 35.3% as opposed to our 66.2%, cefuroxime sensitivity of 32.7% compared with our 55.9%, indicating the variation of drug effects in different geographical areas. Furthermore, a study done in Iran revealed significantly higher resistance (86.6%) to ceftriaxone (Mehrgan *et al*., 2010) than that observed in our study in Karachi and also as reported in Egypt (Ashour and El-Sharif, 2009).

Combination antibiotics showed varying efficacies: piperacillin.tazobactam: 95.7%; cefoperazone.sulbactam: 95.8% and cotrimoxazole (Septran): 43.1 %. The results of cotrimoxazole effectiveness were noted to be currently improved as compared to the previously reported 6.52% by Ullah *et al*. (2009). Our study also underlined the current efficacy of piperacillin.tazobactam on our isolates for years 2009-2010 to have only 2.1% resistance, compared to the resistance (66.8%) for years 2005-2007 reported in Iran (Mehrgan *et al*., 2010). This possibly indicates evolutionary pressure encouraging the survival of resistant strains based on the extent of drug prescription by doctors during the period.

Our figures showing sensitivity of isolates to fosfomycin (77.5%) is lower than that recently reported (86.96%) in Rawalpindi (Khan *et al*., 2010), while the Amino-glycosides showed improved efficacy than that reported previously in India, Pakistan and Egypt (Ghanshyam *et al*., 2002; Ullah *et al*., 2009; Ashour and El-Sharif, 2009). Amikacin indeed showed an efficacy as high as 89.4% in our study while gentamicin exerted only 35.4% effectiveness; certainly, also noted in our scrutiny was one isolate that was resistant to all antibiotics except amikacin. Gentamicin was less effective than the susceptibility figure (43.4%) reported at Rawalpindi (Khan *et al*., 2010) and 50.4% accounted in Egypt (Ashour and El-Sharif, 2009). These significant differences may be attributed to selective pressures by drugs in different regions (Ullah *et al*., 2009). Our results for amikacin sensitivity were in agreement with that reported by Akhtar (2010) and Hawser *et al*. (2011) but in a report from neighboring Iran, the resistance to the drug was 72.8% (Mehrgan *et al*., 2010) as opposed to our 4.4%.

The 5-fluoroquinolone, ciprofloxacin also exerted a higher effectiveness (62.5%) than that reported by Ullah *et al* and Ashour *et al*. This may be due to a withdrawal of use of these antibiotics prior to the years of study. On the other hand, doxycycline, a tetracycline, in our data exerted poor effectiveness (11.5%), also noted in Iran (2%) in 2010 (Mehrgan *et al*., 2010), suggesting excessive use of the drug, inexpensive and easily available, in both areas.

As established in nature that females are more prone to UTIs because of a shorter urethra, 80.7% cases in our study were females which corroborates the results in other reports (Ram *et al*., 2009). However, previous reports show that Klebsiella is isolated most frequently from elderly or very young patients (Mehrgan *et al*., 2010), but in our survey, most of the cases i.e. 31.7% (*n*=497) were aged 16-30.

Nosocomial pathogens are especially multi-drug resistant due to increased selective pressure of antibiotics (Ullah *et al*., 2009). Sources of MDR Klebsiella include meat (Neslihan *et al*., 2011) and particularly catheterization and instrumentation in hospitals (Khan *et al*., 2010). It has been proposed that resistance in microorganisms to antibiotics emerges as rapidly as within 5 years of introduction of a new antibiotic as a therapeutic drug (Bashir *et al*., 2007).

This inspection of the antibiotic resistance profile of *K. pneumoniae* isolates emphasizes the need for implementation of the commonly-accepted but poorly-implemented concept of avoiding the misuse of antibiotics and adherence to antibiotic control policies. Sorely needed is to minimize the emergence of resistant strains before prescription malpractice leads us back to the therapeutic dead-end of pre-antibiotic era. Experts have advised establishment of "antibiotic stewardship index" to gauge the proportion of a country's gross-domestic product that is spent in publically-funded health programmes (Walsh and Toleman, 2012).

REFERENCES

Abhilash KP, Veeraraghavan B and Abraham OC (2010). Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* spp. in a tertiary care teaching hospital in south India. *J. Assoc. Physicians India*, **58**(Suppl.): 13-17.

Akhtar N (2010). Hospital acquired infections in a medical intensive care unit. *J. Coll. Physician's Surg. Pak*., **20**: 386-390.

Amin A, Ghumro PB, Hussain S and Hameed A (2009). Prevalence of antibiotic resistance among clinical isolates of *Klebsiella pneumonia* isolated from a Tertiary Care Hospital in Pakistan. *Mal. J. Microbiol*., **5**: 81-86.

Ashour HM and El-Sharif A (2009). Species distribution and antimicrobial susceptibility of gram-negative aerobic bacteria in hospitalized cancer patients. *J. Transl. Med.*, **7**: 14.

Bashir A, Mujahid TY and Jehan N (2007). Antibiotic resistance profile: Isolation and characterization of clinical isolates of S*taphylococci* from patients with community acquired skin infections. *Pak. J. Pharm. Sci*., **20**: 295-299.

Bush K (2010). Bench-to-bedside review: The role of beta-lactamases in antibiotic-resistant Gram-negative infections. *Crit. Care*, **14**: 224.

Farooqi BJ, Shareeq F, Rizvi QK, Qureshi HS and Ashfaq MK (2000). Changing pattern of antimicrobial susceptibility of organisms causing community acquired urinary tract infections. *J. Pak. Med. Assoc*., **50**: 369-373.

Ganatra HA and Zaidi AK (2010). Neonatal infections in the developing world. *Semin. Perinatol.,* **34**: 416-25

Gootz TD (2010). The global problem of antibiotic resistance. *Crit. Rev. Immunol.*, **30**: 79-93.

Hawser SP, Bouchillon SK, Lascols C, Hackel M, Hoban DJ, Badal RE, Woodford N and Livermore DM (2011). Susceptibility of *Klebsiella pneumoniae* Isolates from Intra-Abdominal Infections, and Molecular Characterization of Ertapenem-Resistant Isolates. *Antimicrob. Agents Chemother*., **55**: 3917-3921.

Hirsch EB and Tam VH (2010). Detection and treatment options for *Klebsiella* pneumoniae carbapenemases (KPCs): An emerging cause of multidrug-resistant infection. *J. Antimicrob. Chemother.*, **65**: 1119-1125.

Johnson MT, Reichley R, Hoppe-Bauer J, Dunne WM, Micek S and Kollef M (2011). Impact of previous antibiotic therapy on outcome of Gram-negative severe sepsis. *Crit. Care Med.*, **39**: 1859-1865.

Khan BA, Saeed S, Akram A, Khan FB and Nasim A (2010). Nosocomial uropathogens and their antibiotic sensitivity patterns in a tertiary referral teaching hospital in Rawalpindi, Pakistan. *J. Ayub Med. Coll. Abbottabad*, **22**: 11-12.

Khan SW and Ahmed A (2001). Uropathogens and their susceptibility pattern: a retrospective analysis. *J. Pak. Med. Assoc.*, **51**: 98-100.

Kumhar GD, Ramachandran VG and Gupta P (2002). Bacteriological Analysis of Blood Culture Isolates from Neonates in a Tertiary Care Hospital in India. *J. Health Popul. Nutr*., **20**: 343-347.

Lagamayo EN (2008). Antimicrobial resistance in major pathogens of hospital-acquired pneumonia in Asian countries. *Am. J. Infect. Control*, **36**: S101-S108.

Mumtaz S, Ahmad M, Aftab I, Akhtar N, ul Hassan M and Hamid A (2007). Extended spectrum beta-lactamases in enteric gram-negative bacilli: Related to age and gender. *J. Ayub Med. Coll. Abbottabad.*, **19**: 107-111.

Mushtaq A, Abid M and Qureshi MA (2012). Is prophylactic antibiotic treatment justified in neonates? *J. Pak. Med. Assoc*., **62**: 856-57

Neslihan G, Sumru C and Emel Y (2000). Virulence Properties of Extended Spectrum β-Lactamase-Producing *Klebsiella* Species in Meat Samples. *J. Food Prot.*, **74**: 559-564.

Shah RK, Singh YI, Sanjana RK, Chaudhary N and Saldanha D. Study of Extended spectrum beta-lactamases (ESBLs) producing *Klebsiella* species in various clinical specimens: A preliminary report. *J. Coll. Med. Sci. Nepal,* **6**: 19-23.

Ullah F, Malik SA and Ahmed J (2009). Antimicrobial susceptibility pattern and ESBL prevalence in *Klebsiella pneumoniae* from urinary tract infections in the North-West of Pakistan. *Afr. J. Microbiol. Res.*, **3**: 676-680.

Walsh TR and Toleman MA (2012). The emergence of pan-resistant Gram-negative pathogens merits a rapid global political response. *J. Antimicrob. Chemother.,* **67**: 1-3

Yoon JE, Kim WK, Lee JS, Shin KS and Ha TS (2011). Antibiotic susceptibility and imaging findings of the causative microorganisms responsible for acute urinary tract infection in children: A five-year single center study. *Korean J. Pediatr*., **54**: 79-85.