In vitro susceptibility pattern of clinically isolated *klebsiella pneumoniae* against beta-lactams, carbapenems, aminoglycosides and quinolones.

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Abstract

Objective: To find the susceptibility pattern of K. pneumoniae against carbapenems, beta-lactams, quinolones and aminoglycosides.

Study setting: The study was conducted at Department of Microbiology in Tertiary Care Hospital of Lahore, from July 2016 to December 2019.

Materials and methods: A total number of 34,381 samples were collected from several wards of the hospital and processed for microbiological analysis. The organisms were identified by using different microbiological techniques. Antimicrobial resistance profile was determined by Kirby-Bauer disc diffusion method.

Results: The positive culture yielded 549 K. pneumoniae. There were 351 (63.9%) male and 198 (36.1%) female patients. The highest number of *K. pneumoniae* was isolated from blood samples 236 (43.0%).

The highest sensitivity of *K. pneumoniae* was seen with imipenem 373 (67.9%) followed by 241 (43.2%) to meropenem, 231 (42.1%) to sulbactam/cefoperazone, 227 (41.3%) to amikacin. Less sensitivity of *K. pneumoniae* was 194 (35.3%) to piperacillin/tazobactam,102 (18.6%) to ciprofloxacin, 66 (12.0%) to moxifloxacin, 50 (9.1%) to co-amoxiclav, 34 (6.2%) to ceftazidime, 31 (5.6%) to cefotaxmine, 30 (5.5%) to ceftriaxone, cefuroxime, 24 (4.5%) to cefixime.

Conclusion: Good infection control practices can help to control the spread of *K. pneumoniae* among the paediatric patients. Carbapenems were found to be more effective in treating K. pneumoniae.

Keywords: Microbiological, Moxifloxacin, Sulbactam/Cefoperazone, Co-amoxiclav.

Abbreviations

APGAR: Appearance, Pulse, Grimace, Activity, and Respiration; CI: Confidence Interval; AOR: Adjusted Odds Ratio; COR: Crudes Odd Ratio; WHO: World Health Organization; ANC: Antenatal Care; C/S: Cesarean Section; SVD: Spontaneous Vaginal Delivery.

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Introduction

K. pneumoniae isGram-negative bacteria that has become a well recognize cause of nosocomial infections [1]. *Klebsiella* are opportunistic pathogens belong to the Enterobacteraciae family of bacteria which can cause neonatal enteritis, meningitis, urinary tract infections, bacteremia and sepsis [2]. *K. pneumoniae* is one of the most common cause of hospital acquired infections. It is also important agent of community acquired infections. *Klebsiella*infections spread via respiratory cause pneumonia or blood stream infections. *Klebsiella* is nosocomial pathogen and can infect the patients on ventilators, catheters and post-surgical wounds.

The worldwide prevalence of *K. pneumoniae* has been reported in France (2.7%), Germany (5.4%), Italy (3.5%), Canada (5.4%) and United States (5.8%). *K. pneumoniae* causes chest infections and severe bronchopneumonia with lung abcesses. Infections are opportunistic, occurring in those with existing chest disease or diabetes mellitus or malnourished persons [3].

Beta-lactam antibiotics are broad class of antibiotics, consisting of all antibiotic agents that contain a beta-lactam ring in their structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. Mechanisms of resistance are found with in bacteria either intrinsically or they may be acquired. Acquired resistance mechanisms are attained by bacteria through mutations or mechanisms of horizontal gene transfer such as conjugation, transduction and transformation [4]. MBL were first identified nearly 35 years ago in a type of Bacillus cereus and exhibited interesting properties, including cephalosporinase activity and inhibition by EDTA.

Carbapenems are the class of beta-lactam antibiotics with a broad-spectrum antibacterial activity. They have a structure that renders them highly resistant to most beta-lactamases. Carbapenems are often used as last resort antibiotics for treating infections caused by multidrug resistant (MDR) Gram negative bacilli [5]. The resistance to carbapenems is now a major world-wide issue. According to Ambler classification, carbapenems hydrolysing enzymes belong to class B. The beta-

lactamases in the class B require one or two zinc ions for their full catalytic activity and these enzymes are therefore called metallo-beta-lactamases (MBLs). Metallo-beta-lactamases are considered to be more serious than other resistant mechanisms because MBLs can almost hydrolyse all beta-lactam antibiotics [4].

Aminoglycosides contain a portion of an amino-modified glycoside (sugar) and inhibit protein synthesis.The term can also refer more generally to any organic molecule that contains aminosugar substructures. Aminoglycoside antibiotics display bactericidal activity against Gram-negative aerobes and some anaerobic bacilli where resistance has not yet arisen, but generally not against Gram-positive and anaerobic Gram-negative bacteria [5].

The quinolones are a family of synthetic broad-spectrum antibacterial drugs. Quinolones are broad spectrum antimicrobial agents which are used for the treatment of various infections. It has excellent activity against clinically isolated *Klebsiella* and now has become less effective due to their extensive use. Early studies have shown that quinolones resistance arises due to mutation in topoisomerase subunits as well as changes in expression of efflux pumps and porins which control the accumulation of these agents inside the bacterial cell. Antibiotics in this group are nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin, levofloxacin and gatifloxacin [6].

Antimicrobial susceptibility pattern determined by using disk diffusion antimicrobial susceptibility testing, (CLSI recommended) inhibitory zones diameters around the antibiotic disks, results interpreted as sensitive, intermediate or resistant. For gram positive bacteria, antibiotic disks used are: oxacillin, cephalothin, clindamycin, ciprofloxacin and meropenem [7]. For gram negative bacteria antibiotics are used: amikacin, gentamicin,cefepime, ceftazidime, ciprofloxacin, levofloxacin, aztreonam, imipenem, meropenem, piperacillin and tazobactam.

The results of susceptibility pattern of *K. pneumoniae* will provide guideline for treatment and choice of best antibiotics against K. pneumoniae.

The objectives of this study were to determine the:

- Frequency of K. pneumoniae.
- Susceptibility pattern of K. pneumoniaeagainstbeta-lactams, carbapenems, aminoglycosides and quinolones.

Materials and Methods

Study design and settings

Descriptive cros-sectional study was conducted at Department of Microbiology in Tertiary Care Hospital of Lahore.

Sample size: 34,381

Sampling technique: Consecutive sampling

Sample selection: Following specifications were used to include or exclude the samples

Inclusion criteria:

- Gender: Both male and female.
- All clinical samples processed to microbiology department.

Exclusion criteria:

- All other patients who are not fulfilling the above mentioned criteria are excluded.
- Clotted blood samples.
- When sputum sample mostly contain saliva.
- Improperly labelled samples.

Sample collection: Various clinical samples such as blood, urine, cerebrospinal fluid, pus, tips and respiratory tract samples were collected from different wards of Tertiary Care Hospital of Lahore after an institutional ethical approval.

Sample processing: All samples during study period were further processed for culture as follows

Blood: Blood culture bottles received in lab were incubated at 35-37°C for up to 7 days and routinely inspected twice a day for signs of microbial growth. Growth was evidenced by:

- Turbidity
- Haemolysis
- Clot formation
- Gas production

When there was an indication of growth, these samples were sub-cultured on Blood and MacConkey agar. Plates were incubated aerobically for up to 48 hours. Bottles with no growth were re-incubated up to 7 days and sub-cultured on 7th day.

Peritoneal, pleural fluid and tracheal secretion, Tips, Sputum, Pus, Swabs: Pleural, peritoneal dialysis fluid and tracheal secretion were inoculated on Blood and MacConkey agar plate aerobically at 35-37°C overnight.

CSF: CSF samples were cultured on MacConkey agar aerobically at 35-37°C for the isolation of *Klebsiella* pneumoniae and also on Blood and Chocolate agar in a carbon dioxide enriched atmosphere at 35-37°C for up to 48 hours for the isolation of other bacteria.

Urine: Urine specimen was inoculated on CLED agar plate. Calibrated wire loop of 2µl size was used to inoculate the agar plate. The plates were incubated overnight at 37°C for 24 hours.

Identification of bacteria

Gram staining: gram staining was performed according to manufacturer instructions.

Bio chemical test

Oxidase Test: A piece of filter paper was placed in a clean petri dish and two or three drops of freshly prepared oxidase reagent were added. With a wooden stick a colony of test oragnisms was streaked on the filter paper. No colour

development within 10 seconds showed a negative test while a positive test was indicated by the appearance of purple colour.

API 10S: API 10S is used for the identification of Enterobacteriaceaeand other Gram negative bacteria. It is a fast identification system which is based on 10 biochemical reactions carried out in mini wells which contain substrate in them and a database. The wells containing dehydrated substrate are present on API 10S strip.

Antimicrobial susceptibility testing: Antibiotic sensitivity testing was performed to determine the sensitivity pattern of test organisms by using Kirby-Bauer Disk Diffusion method. Different antibiotics were tested which included amikacin (AK), cefuroxime (CXM), cefixime (CFM), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), sulbactam/ cefoperazone (SCF), ciprofloxacin (CIP), meropenem (MEM), imipenem (IPM), and piperacillin/tazobactam (TZP), co-amoxiclav (AMC), moxifloxacin (MXF).

Results

A total number of 34,381 samples were processed for microbiological analysis, out of which 3910 (11.3%) were positive cultures. Among the positive cultures, 549 (14.04%) were *K. pneumoniae* (Table 1).

Organisim	Frequency	Percentage
K. pneumoniae	549	14.04
Other bacteria	3361	85.96

Table 1. *Frequency of K. pneumoniae among the various positive culture (n=3910).*

K. pneumoniae were isolated from both males and females. Gender wise frequency distribution in males and females was 351 (63.9%) and 198 (36.1%) respectively (Figure 1).

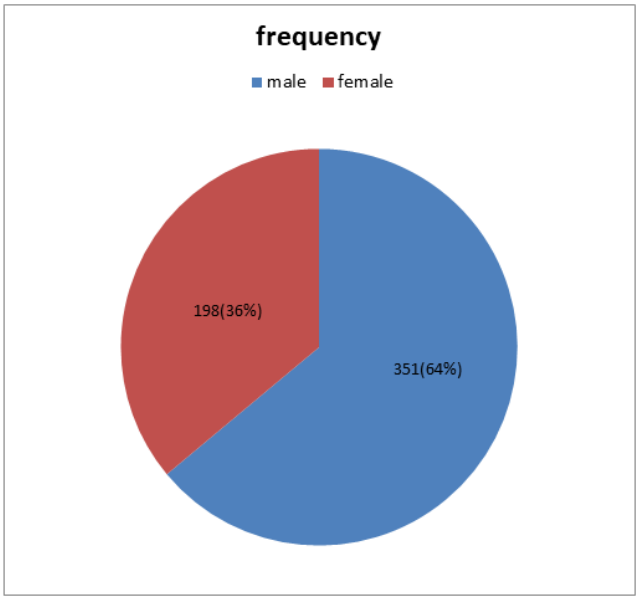

Figure 1. *Gender wise frequency of K. pneumoniae (n=549).*

The highest frequency of clinically isolated *K. pneumoniae* from blood was 236 (43.0%) followed by 121 (22.0%) from urine, 83 (15.1%) from pus, 30 (5.5%) from cerebrospinal fluid, 28 (5.1%) from bronchial specimens,17 (3.1%) from endotracheal tube, 11 (2.0%) from central venous tips, 11 (2.0%) from sputum, 4 (0.7%) from pleural fluid, 4 (0.7%) from ear swabs, 3 (0.5%) from peritoneal dialysis catheter and 1 (0.2%) from ascetic fluid (Table 2).

Specimen	Frequency	Percent
Blood	236	43.0
Urine	121	22.0
Pus swab	83	15.1
Ear swab	4	.7
Bronchus swab/ aspirate	28	5.1
Sputum	11	2.0
E.T.T	17	3.1
C.V.P Tip	11	2.0
C.S.F	30	5.5
Ascitic fluid	1	.2
Pleural fluid	4	.7
P. D. cathter tip	3	.5

Table 2. *Distribution of K. pneumoniae in various specimens (n=549).*

Highest of *K. pneumoniae* from various wards was as follows: medical units 114 (20.82%), neonatal emergency 93 (16.9%), followed by nephrology 88 (16.0%), neonatal unit 50 (9.1%), plastic surgery ward 40 (7.3%), surgical follow up 37 (6.7%), surgical intensive care units 36 (6.6%), oncology 33 (6%), neurology 29 (5.3%), orthopedic 8 (1.5%), gastrology 6 (1.1%) (Table 3).

Wards	Frequency	Percent
NNU	50	9.1
NNE	93	16.9
PSW	40	7.3
SICU	36	6.6
Medical units	114	20.82
Orthopaedic	8	1.5
H/Onco	33	6
Neurology	29	5.3
Cardiology	15	2.7
Nephrology	88	16
Gastrology	6	1.1
S. Fup	37	6.7

Table 3. *Ward wise frequency distribution of K. pneumoniae (n=549).*

The highest sensitivity of *K. pneumoniae* was seen with imipenem 373 (67.9%) followed by 241 (43.2%) to meropenem, 231 (42.1%) to sulbactam/cefoperazone, 227

(41.3%) to amikacin. Less sensitivity of *K. pneumoniae* was 194 (35.3%) to piperacillin/tazobactam,102 (18.6%) to ciprofloxacin, 66 (12.0%) to moxifloxacin, 50 (9.1%) to co-amoxiclav, 34 (6.2%) to ceftazidime, 31 (5.6%) tocefotaxmine, 30 (5.5%) to ceftriaxone, cefuroxime, 24 (4.5%) to cefixime. There were two additional antibiotics norfloxaxin and pipemedic acids were applied on urinary isolates. The sensitivity of *K. pneumoniae* to these antibiotics was 36 (29.5%) and 25 (20.5%) to norfloxaxin and pipemedic acid respectively (Table 4).

Antibiotics	Sensitive	Intermediate Sensitive n (%)	Resistant
	n (%)		n (%)
Amikacin (AK)	227 (41.3)	23 (4.2)	299 (54.5)
Co-amoxiclav (AMC)	50 (9.1)	5 (0.5)	494 (90.0)
Cefuroxime (CXM)	24 (4.5)	2 (0.4)	523 (95.3)
Cefixim (CFM)	24 (4.5)	2 (0.4)	523 (95.3)
Cefotaxmine (CTX)	31 (5.6)	0 (0.0)	518 (94.4)
Ceftazidime (CAZ)	34 (6.2)	4 (0.7)	511 (93.1)
Ceftriaxone (CRO)	30 (5.5)	1 (0.2)	518 (94.4)
Ciprofloxacin (CIP)	102 (18.6)	61 (11.1)	386 (70.3)
Sulbactum/ Cefoperazone (SCF)	231 (42.1)	32 (5.8)	285 (51.9)
Piperacillin/ Tazobactam (TZP)	194 (35.3)	50 (9.1)	304 (55.4)
Moxifloxacin (MXF)	66 (12.0)	4 (0.7)	479 (87.2)
Meropenem (MEM)	241 (43.9)	20 (3.6)	288 (52.5)
Imepenem (IPM)	373 (67.9)	74 (13.5)	102 (18.6)
Norfloxaxin (NOR)	36 (29.5)	2 (0.01)	84 (68.8)
Pipemedic acid (PIP)	25 (20.5)	2 (0.01)	95 (77.86)

Table 4. *Antibiotics, Sensitive, Intermediate Sensitive and Resistant.*

Discussion

K. pneumoniae is one of the most common cause of hospital acquired infections. It is also important pathogen of community acquired infections. Cephalosporins, fluoroquinolones, aminoglycosides and carbapenems are effective for treating infections caused by *Klebsiella*. The continuous use of antibiotics and the resulting difficulty in antibiotic choice has led to an increasing rate of antibacterial resistance among Gram negative bacteria especially K. pneumoniae. Carbapenems are broad spectrum antibiotics that are often used as last resort treatments for resistant Gram negative infections caused by extended spectrum beta-lactamase (ESBL) producing K. pneumoniae.

A total number of 34,381 samples were processed for microbiological analysis, out of which 3910 (11.3%) were positive cultures. Among the positive cultures, 549 (14.04%) were K. pneumoniae. This study demonstrated the sensitivity pattern of *K. pneumoniae* in paediatric patients. The highest sensitivity was seen with imipenem 373 (67.9%). Lowest sensitivity was seen with cefuroxime 24 (4.5%).In this study, among the quinolones, sensitivity with amikacin was 194 (35.3%). Shawkey et al., conducted a study at Alexandria main university hospital, Queen Nazli Children Hospital found 22.3% sensitivity rate which is lower than this study. In another study, the sensitivity rate of ciprofloxacin and moxifloxacin was 12.9% and 17.2% which is close to the present study.

A previous study conducted by Haung et al., in China showed 29% sensitivity to amikacin which is contradictory to present study. They reported antimicrobial sensitivity pattern of 23% to ciprofloxacin , 27% to ceftazidime, 29% to cefotaxmine, 27% to ceftriaxone and 2% to piperacillin/tazobactam. According to the present study, a wide contradiction is present. Another study conducted by Raeiet al.,. in Iran reported 36% sensitivity to ciprofloxacin, 50.5% to ceftazidime, 50.5% to cefotaxmine, 47.9% to ceftriaxone and 18.3% to piperacillin/tazobactam. These results were contradictory to the present study.

The present study revealed highest sensitivity to imipenem while a previous study conducted in India showed no activity to imipenem. Manjula et al., in India reported 36.5% sensitivity to ciprofloxacin, 63.4% to cefotaxmine and 39% to piperacillin/tazobactam. The sensitivity pattern of piperacillin/ tazobactam was approximately close to the present study. According to Kumar, in India, highest sensitivity was seen with amikacin 92.7% that was not resemble to the present study. Other results were ciprofloxacin 51.2%, ceftazidime 44%, ceftriaxone 47.2%, cefuroxime 4.5%, co-amoxiclav 11.5% and norfloxacin 44.1 %.

Dhanraj, reported that *K. pneumoniae* highly sensitive to ciprofloxacin among the quinolones while this study illustrated that among quinolones, amikacin was highly sensitive antibiotic drug. Another study in India reported by Sikarwar and Batra, revealed highly sensitive *K. pneumoniae* to ciprofloxacin among quinolones. Amikacin was also effective but less than ciprofloxacin while the present study demonstrated amikacin was more effective than ciprofloxacin.

In India, a study was conducted by Kumarasany et al.,. on resistance mechanism of *K. pneumoniae* showed same results as reported in U.K., Haryana and Chanii. The results included no activity to imipenam, amikacin, cefotaxmine, ceftazidime and piperacillin/tazobactam. The sensitivity of ciprofloxacin and meropenem was 8% and 3% respectively while the present study showed that imipenem and meropenem were highly effective drugs.

Ullah et al., conducted a research in Pakistan, in which ESBL producing strains were highly sensitive to carbapenems. Meropenem was 100% and imipenem was 94% sensitive. Non-ESBL producing strains were also highly sensitive to carbapenems. The sensitivity was meropenem 93%, imipenem 86%, amikacin 63%, ciprofloxacin 41%, ceftriaxone 32%, ceftazidime 28% and co-amoxiclav 17%. The present study also showed carbapenems were highly effective antibiotics.

Conclusion

It was concluded that *K. pneumoniae* was highly sensitive to imipenem and meropenem and resistant to cefixim and cefuroxime. The study confirmed the sensitivity pattern of *K. pneumoniae* against various classes of drugs in paediatric patients.

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Authors' contributions

YMA, TYA, MG and MT: Approved the proposal with some revisions, developed the design and methodology of the study, literature review, quality evaluation, statistical analysis, data interpretation and drafting of the manuscript.

YMA, AN, YAA and WSS: Wrote the proposal, participated in data collection, and analyzed the data. They were also participated in statistical analysis and interpretation, quality assessment, prepared the final draft of the manuscript. Finally, all authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethical clearance letter has been received from the research and review committee at Debre Marks University. Additionally, prior to beginning data collection permission was obtained from the hospital authority. Finally, an informed written consent was received from each participant mothers after explaining to them the research objectives. The participants were briefed on the study's purpose, procedures, potential risks, and benefits. In addition, the participants were told that failure to agree to or withdraw from the study would not change or endanger their access to treatment.

Consent for publication

Not applicable.

Competing interests

The authors state no competing interests.

References

1. Mueller L, Ottiger C, Demord A, et al. Multiple colonization with carbapenem-resistant Gram-negative bacteria acquired in India and transferred to Switzerland. Infection. 2019;47:669-71.
2. Khan HA, Ahmad A, Mehboob R. Nosocomial infections and their control strategies. Asian J Trop Biomed. 2015;5:509-14.
3. Maltezou HC, La-Scola B, Astra H, et al. Mycoplasma pneumoniae and Legionella pneumophila in community-acquired lower respiratory tract infections among hospitalized children: diagnosis by real time PCR. Sca J Inf Dis. 2004;36:639-42.
4. Revelas A. Healthcare–associated infections: A public health problem. Nig Med J. 2012;53:59.
5. Jones ME, Draghi DC, Thornsberry C, et al. Emerging resistance among bacterial pathogens in the intensive care unit–a European and North American Surveillance study (2000–2002). Annals of Clinical Microbiology and Antimicrobials. 2004;3:1-1.
6. Gadkowski LB, Stout JE. Cavitary pulmonary disease. Clini Microbiol Rev. 2008;21:305-33.
7. Bush K, Macielag MJ. New β-lactam antibiotics and β-lactamase inhibitors. Exp Opin Ther Pat. 2010;20:1277-93.

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