Original Article

Imipenem Hope and Threat From Carbapenemase Enzyme Producing Multi-Drug Resistant (MDR) Klebsiella Pneumoniae

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ABSTRACT

Objective: To evaluate the in–vitro efficacy of various antibiotics against Klebsiella pneumoniae by paper disk diffusion method and detection of enzyme carbapenemase of the isolates.

Study Design: Prospective study

Place and Duration of Study: This study was conducted at the Department of Pathology and Microbiology, Indus Medical College Tando Muhammad Khan, for the period of 9 months from Sep 2018 to June 2019.

Materials and Methods: Strains were isolated and preserved in Nutrient agar slope in Beijou bottles, followed by subcultures on blood agar plate. Panel of antimicrobial agents e.g. Imipenem, Aztreonam, Gentamicin, Amikacin, Ceftazidime, ciprofloxacin, Cotrimoxazole, Ceftriaxone, Cefoxitin, Keflex, Piperacillin and Ampicillin were used.

Results: 104 (95%) strains were sensitive to Imipenem, 48 (44%) sensitive to Amikacin, 45 (41.2%) to Ciprofloxacin, and 32 (29.32%) sensitive to Cotrimoxazole. While 19 (17.4%) were sensitive to Ceftazidime, 18 (16.5%) to Ceftriaxone, and 21 strains showed Carbapenemase enzyme positive activity.

Conclusion: Imipenem and other antimicrobial agents showed increased resistance to the organism. It will not be without risk of treatment failure and adverse impact on cost-effectiveness.

Key Words: Klebsiella pneumoniae, Imipenem, Carbapenemase, Multi-drug resistant

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INTRODUCTION

Imipenem one of the most popular antibiotic of the group carbapenem is used as an intravenous beta-lactam antibiotic, was formulated and presented by Kenneth Wildonger, Burton Christensen, and William Leanza in the mid-1970s, these scientists worked for MERCK and company.[1] Most of the beta-lactam drugs are antagonized and thereby made ineffective by beta-lactamase enzymes. While imipenem is stable against beta-lactamase enzymes as it is not neutralized by many bacteria which are resistantto antibiotics, including klebsiella pneumoniae.[2] hence proved to be significant part in treating nosocomial infections that are not easily cured with other antibiotics that are available.[3,4]

In 1975 Imipenem became patented and in 1985 was sanctioned for medical consumption.[3] Through lengthy trial-and-error research, it was found that a more stabilizedform thienamycin (natural product), thatwascreated by the micro-organism Streptomyces cattleya. Thienamycin has natural antibacterial natural activity, but being unsteady in aqueous solution, was not effective and viable agent to use in the patients.[5] Imipenem possesses a wide spectrum of anti-bacterial activity against both the aerobic and anaerobicorganism, including Gram-positive as well as Gram-negative bacteria.[6]

The anti-microbial activity of Imipenem is due to its antimicrobial action i.e. preventing cell wall synthesis of various Gram-positive and Gram-negative bacteria. It remains very stable in the presence of beta-lactamase enzymes i.e. (both penicillinase and cephalosporinase) raised as a result ofa few bacteria, moreoverit strongly inhibits beta-lactamases from a few Gram-negative bacteria which are found to be resistant to many of the beta-lactam antibiotics.[7]

Klebsiella pneumonia, one of the Gram-negative bacteria which has been found to causemany infections among humans; to mention few of them are pneumonia,

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septicemia, wound infections of many types including surgical one, and meningitis. Progressively, Klebsiella bacteria have evolved to acquire antimicrobial resistance, nearly allrelated to the family of antibiotics recognized as carbapenems. Klebsiella bacteria, are commonly found within the human intestines (where, surprisingly, these bacteria do not cause any disease). Inaddition,same bacteria can be found in individual's stool (feces). In hospitals and other healthcare settings institutions, Klebsiella infections usually occur among patients who are sick and are under treatment for some other conditions. Even patients, who require intensive care or on life-saving devices like ventilators, or having intravenous catheters, and also the patients who are prescribed to take long doses of certain antibiotics are vulnerable to infections by Klebsiella infections. Whereas, fit and healthy people normally do not acquire Klebsiella infections.

Over the period, few Klebsiella bacteria became extremelyresistant to antimicrobial agents. Once bacteria like Klebsiella pneumoniae produces an enzyme identified as a carbapenemase (well-known for KPC-producing organisms), the family of antibiotics called carbapenems would not be effective in killing the bacteria and cannot treat the infection completely. Klebsiella bacteria are mostly found in intestinal tract as a normal flora and these species belong to family of Enterobacteriaceae that can develop into carbapenem-resistant. CRE (carbapenem-resistant Enterobacteriaceae). CRE are a family of microbes that are hard to treat since they have high-level of resistance to antibiotics. Regrettably, carbapenem antibiotic oftenare the last resort or drug of choice curtail infections produced by Gram-negative bacteria that are resistant to many other antimicrobials.[8]

Resistance to antimicrobial agents (AMR) led to increased mortality rate from unsuccessful treatments and compounded the cost of medical expenditure at many healthcare settings. While accurately predicting the exact public health risk and the associated cos may not be easily calculated because of multiple interplaying factors, but undoubtedly the rise to antibiotic resistance and emergence of mutationis a global threat that may lead to undesirable consequence i.e. pandemic. Hospital acquired infections are one of the major health problems globally due to increased morbidity and mortality. Patients staying in hospitals for prolonged periods aremore vulnerable to Klebsiella pneumoniae infections.

Timely evaluation, detection and appropriate treatment in the management of different infections are necessary to reduce the morbidity and mortality rate of such patients. Primary aim of this study is to assess the in vitro efficacy of various antibiotics against Klebsiella pneumoniae by paper disk diffusion method isolated from various clinical specimens and to ascertain appropriate antimicrobial agent in the clinical settings.

Background of the study: Researchers observed individual patients' case thoroughly and performed regular routine lab test culture and sensitivity twice at Indus Medical College Hospital, Tando Mohammad Khan. In both instances the effect and treatment of imipenem drug (Carbapenem group) failed. To find out the cause of treatment failure, an additional test to detect Carbapenemase enzyme was carried out and it was concluded that in the patient this enzyme was found due to Klebsiella pneumoniae, producing enzyme carbapenemase. This became the basis for further study to investigate the issue in detail so as to find which the drug was not effective to kill the microbial agents.

MATERIALS AND METHODS

It was a prospective observational study conducted at Department of Microbiology, Indus Medical College Tando Muhammad Khan. The study was carried out for the period of 9 months (Sep 2018 to June 2019). A total of 109 strains of Klebsiella pneumoniae were studied. The strains were isolated from various clinical specimens. These isolates were preserved in Nutrient agar slope in Beijou bottles that were labeled and refrigerated. At the time of study organisms were sub-cultured on Blood agar plate. The identification criteria were: Gram stain i.e. gram-negative bacilli, lactose fermenter, mucoid colonies, non-motile, citrate positive, urease positive, indole negative,(MIU) Motility Indole Urea medium was used. Klebsiella **pneumonia** ATCC 700603was included as a control strain.

Antimicrobial agents used were, Imipenem (IMI), Aztreonam (AZT), Gentamicin (GEN), Amikacin (AK), Ceftazidime (CAZ), Ciprofloxacin (CIP), Cotrimoxazole (TS), Ceftriaxone (CRO), Cefoxitin (Cef), Keflex (KF), Piperacillin (PIP),and Ampicillin (AP). All drugs were tested for their susceptibility.[4]

Results were interpreted according to Kirby – Bauer method. Paper disk diffusion method was adopted using Mueller – Hinton agar and paper disks of antibiotics from **Oxoiddistributors**. The inhibition zone of antibiotics according to **CLSI M7-A10**[4]Sensitive zones of antibiotics was adopted showing in table-1. Due to financial problem only, Imipenem wastested with few strains and control organism by **Eteststrip** (bioMerieux) and showed nearly same results. Klebsiella pneumoniae ATCC 13495.

RESULTS

104 (95.4 %) strains were sensitive to Imipenem,48 (44 %) showed their sensitivity toAmikacin,45 (41.2%) sensitive to ciprofloxacin and 32 (29.32%) were sensitive to Cotrimoxazole. While 19(17.4%) were sensitive to Ceftazidime, 18(16.5%) showed their sensitivity to Ceftriaxone, 18 (16.5%) sensitive to gentamicin, 9 (8.25%) sensitive to Cefoxitin, and 9 (8.25%) were sensitive to Keflex. And there was no

sensitivity against (0%) Piperacillin and ampicillin. The results are shown in the table 1, above. While their in-vitro performance is shown in Table-2.

From above studyusing all antibiotics / drugs used, Imipenem proved to be the drug of choice (95.4%) for Klebsiellapneumoniae, a big hope for multi-drug resistant Klebsiella pneumoniae. We performed another study for detection of carbapenemase enzyme i.e. Boronic acid disk method for carbapenemase detection Kp.[11]

Identification of carbapenemase producers in the clinical research laboratory is of great importance for the finding an effective therapeutic scheme and to suggest ways to effectively place infection control measures**.**

Working solution of the Boronic acid of 20 µl (including Boronic acid 400 µg). was poured on one of each pair of Imipenem disk on impregnated Muller Hinton agar. Imipenem disk plane and Imipenem disk with Boronic acid and agar plates were incubated. On next day. Plates were checked for zone of inhibition.[11]

Table No.1: Antibiotics concentrations and sensitivity zones:

Sr. No.	Antibiotics	Concen-tration Mcg / ml	Sensitive Zone Diameter / mm	Sensitive (%)
1	Imipenem	10 mcg	≥14	95.4
2	Amikacin	30 mcg	≥23	44
3	Ciprofloxacin	5 mcg	≥17	41
4	Cotrimoxazole	30 mcg	≥16	29.35
5	Ceftazidime	30 mcg	≥25	17.4
6	Ceftriaxone	30 mcg	≥18	16.5
7	Aztreonam	30 mcg	≥12	16.5
8	Gentamicin	10 mcg	≥20	16.5
9	Cefoxitin	30 mcg	≥15	8.25
10	Keflex	30 mcg	≥17	8.25
11	Piperacillin	100 mcg	≥15	0
12	Ampicillin	10 mcg	≥15	0

Table No.2: In vitro activity of deferent antibiotics against Klebsiella pneumoniae (n=109)

No	Name of Antibiotic	Total isolates	Sensitive	% Sensitive
1	Imipenem	109	104	95.4 %
2	Amikacin	109	48	44 %
3	Ciprofloxacin	109	45	41.2 %
4	Cotrimoxazole	109	32	29.35 %
5	Ceftazidime	109	19	17.4 %
6	Ceftrixone	109	18	16.5 %
7	Aztreonam	109	18	16.5 %
8	Gentamicin	109	18	16.5 %
9	Cefoxitin	109	9	8.25 %
10	Kaflax	109	9	8.25 %
11	Pipracillin	109	0	0 %
12	Ampicillin	109	0	0 %

Interpretation: The deference of 5 mm zone more was considered positive for carbapenemase producers.

Control strains: K. pneumonia BAA 1705 (positive control) and K. pneumonia BAA 1706 (negative control) both were used.

All (n=109) strains of Klebsiella pneumoniae were tested for carbapenemase enzyme, 21 strains showed Carbapenemase enzyme positive, that was **Threat** on our hope for treatment of Multi-drug resistant Klebsiella pneumoniae. Fig-2.

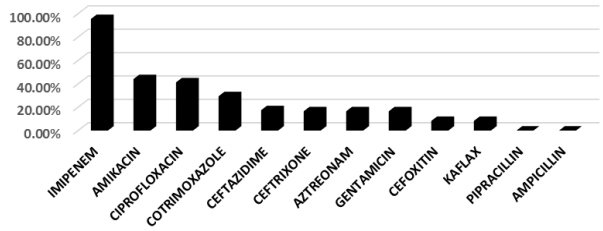

Figure No.1: Klebsiella Pneumonia Antibiogram

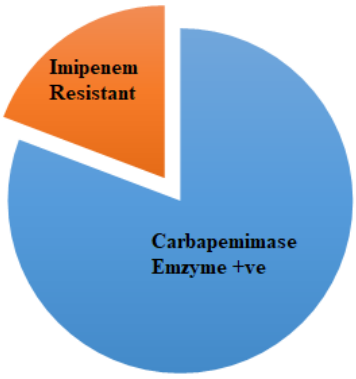

Figure No.2: Difference between imlpenem resistant and carbapemimase emzyme +ve Klebsiella Pneumoniae (n=109)

DISCUSSION

Klebsiella pneumoniae is the major and primary cause of hospital–acquired infections. Due to continuously emerging resistance, treatment is a big challenging encountered by physician. Various antimicrobial agents are used in different settings, resistance to antibiotic drugs in various strains of Klebsiella pneumoniae occurs through a number of mechanisms**,** such as production of enzyme carbapenemase. One of the serious obstacles to any antimicrobial therapy of contagious infections caused by Gram-negative organisms is the presence of carbepenemases. Plasmid-mediated serine carbepenemases and Metallo-beta-lactamases such as Klebsiella pneumoniae carbapenemase endanger the usage of nearly all presently available beta-lactams including carbapenems.[10]

To find and detect the organisms that produce carbepenemases can be very difficult task, since their active presence not always bring about a resistant phenotype on conventional disc diffusion or even automated computerized testing techniques for example Phoenix and micro-scan. These automated testing

techniques can detect MICs but cannot detect carbapenemase enzyme. Often these enzymes are associated with laboratory research reports of false susceptibility carbapenems that carries potential serious harm. Furthermore, nearly all laboratories do not have technical facilities to detect carbepenemases. This perhaps can be due to the lack of proper guidelines or a deficient knowledge and skill.

Since routine sensitivity tests may not be reliable, specialized techniques are advised to ascertain the severity and pattern of associated resistance. The research described in this article identifies the standard methodological analyses to detect various types of carbepenemases that may be implemented by laboratories engaged in the task of ascertaining cause of Gram-negative antimicrobial resistant bacteria. Therefore, the fast detection of carbapenemase production is essential to prevent their dispersion by effectively starting infection control measures of hospital acquired infections.[11]

CONCLUSION

To conclude the study between routine antibiotic sensitivity of Imipenem with Klebsielle pneumoniae and enzyme detection of Klebsiella pneumoniae was different showing increased percentage-wise resistance that was alarming for treatment of the patients having hospital acquired infections.

This study clearly indicates that use of Imipenem, against Klebsiella pneumoniaeon routine sensitivity results will not be without the risk of treatment failure consequently endangering the cost-effectiveness.

Author's Contribution:

Concept & Design of Study:	Qandeel Abbas Soomro
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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