ISSN No:-2456-2165

Evaluation of Enzyme Producing K. Pneumoniae and Their Susceptibility to Other Anti-Biotics

[1]Purabi Saha, [3]Anubhav Dubey, Dr. Sanjay Kumar, [4]Roshan Kumar*
[1]Department of Pharmacy, Uttaranchal Institute of Pharmaceutical Sciences, Dehardun,Uttarakhand, India.
2Associate Dean Cum Principal, School of Pharmacy, Carrer Point university, Hamirpur, HP, India.
[3]Assistant Professor, Department of pharmacy, Maharana Pratap College of Pharmacy, Kanpur, Uttar Pradesh, India
[4]Research Scholar, Department of Pharmacology, Dev Bhoomi Institute of Pharmacy and research, Dehradun, Uttarakhand, India

Abstract:-
Objective: Antimicrobial-resistant microbes are now infecting doctors, veterinarians, and other infection control specialists.
Material & Method: Collection of samples and isolation of K. pneumoniae, Anti-microbial Susceptibility pattern, Hodge's modified test, Extraction of the bla KPC2 gene's DNA
Result & Discussion: More than half of the isolates were resistant to both imipenem and meropenem, with four being just resistant to imipenem. Among the 38 carbapenem resistant isolates, 71% had imipenem MICs above CLSI norms, whereas the remaining 79.9% were intermediately resistant. The MIC50 and MIC90 were 0.19-32, 4 and 16 g/ml, respectively (21.05 percent). Eight of the isolates had MHT. The blaKPC-2 imipenem MIC is 4-32 g/ml. Neither colistin nor co-trimaxazole resistant BlaKPC-2 isolates.
Conclusion: Our institution found blaKPC-2 to be a carbapenem resistant K. pneumoniae pathway. Results demonstrated imipenem disc diffusion can quickly identify these isolates. These illnesses and their associated drug resistance demand infection control and antibiotic stewardship measures.

Keywords:- *K. Pneumonia, gene, DNA, Anti-microbial.*

I. INTRODUCTION

It is common in ICU patients and immunocompromised people. Rising MDR K. pneumoniae strains have garnered much attention. [1-3] Contrary to popular belief, carbapenems had the best in vitro effect against ESL-producing K. pneumoniae. [4] CR E. coli has a considerable risk of morbidity and mortality. [5,6] KPC from Klebsiella pneumoniae is a frequent carbapenem resistance mechanism. [7] It's currently a major global health issue. Antimicrobial resistance is thus a global issue. [8] We need to avoid and control KPC-producing microorganisms globally due to present treatment limits. Our lab looked at the prevalence of KPC-producing K. pneumoniae and their antibiotic susceptibility. Antimicrobial-resistant microbes are now infecting doctors, veterinarians, and other infection control specialists. Invading antibiotic-resistant superbugs New drugs to treat drug-resistant bacterial infections are scarce [9]. Klebsiella pneumoniae is quite harmful. Klebsiella pneumoniae is an opportunistic pathogen.

II. MATERIAL & METHOD

- *Collection of samples and isolation of K. pneumoniae*

104 K. pneumonia isolates from university name Sources of staining: blood, urine, wounds. Gram staining, motility, indole synthesis, and citrate consumption.

- *Anti-microbial Susceptibility pattern*

The Clinical and Laboratory Standards Institute (CLSI) recommends using disc diffusion (Kirby-Bauer) to screen for carbapenem resistance in Gram-negative bacteria [(13).] TS (1.25/23.75g), CRO (30g), CAZ (30g), amikacin (30g), ciprofloxacin (5g), ofloxacin (10g), imipenem (10g), meropenem (10g), and colistin (10g) (Mast diagnostics, Merseyside, U.K.) (Mast diagnostics, Merseyside, U.K.). MDR K. pneumoniae was identified as having three or more drug resistances.

- *Hodge's modified test[12]*

All isolates met CLSI requirement for MHT carbapenemase identification. Two inoculums were used: a CLSI standard inoculation (three to five colonies) and a large inoculation (a full 1L loop). Two observers were blinded to the results.

- *Extraction of the bla KPC2 gene's DNA*

From clinical isolates, we used Promega's Wizard® Genomic DNA Purification Kit (Madison, USA). The Nanodrop-ND1000 assessed the DNA's (Thermo Fisher Scientific, Waltham, MA, USA). (ATGTCACTGTATCGCCGTCT-3')[10]. Heterozygosity of bla KPC gene (882 nucleotides). We used an AccuPrime Taq DNA polymerase (Invitrogen/Stratagene, La Jolla, CA). Amplification was completed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. We used 8 l of each PCR product to electrophorese to determine PCR efficiency and fragment size (TAE: 40 mM Tris-acetate and 1 mM EDTA). USA Promega 100-bp DNA ladder Then came ethidium bromide (0.5 g/ml) staining and UV photography (260 nm).[11,12]

- *Method of statistics*

SPSS for Windows was used to analyse the data using descriptive statistics (version 19 SPSS Inc., USA)

III. RESULT & DISCUSSION

In 2021-2022, a NAME University gathered 104 K. pneumoniae isolates from 60 men and 44 women, respectively. Resistance to meropenem was 32.7 percent and imipenem was 31.7 percent. The best antibiotic was Colistin (98.1 percent). 11.5 percent of the 104 isolates were MDR K. pneumoniae. In the inhibitor-potentiated disc approach, 38 (55.1%) of K. pneumoniae isolates developed ESBLs. Initially, meropenem or imipenem resistance was employed to detect carbapenem-resistant isolates, and 38 (36.5%) were. More than half of the isolates were resistant to both imipenem and meropenem, with four being just resistant to imipenem. Among the 38 carbapenem resistant isolates, 71% had imipenem MICs above CLSI norms, whereas the remaining 79.9% were intermediately resistant. The MIC50 and MIC90 were 0.19-32, 4 and 16 g/ml, respectively (21.05 percent). Eight of the isolates had MHT. The blaKPC-2 imipenem MIC is 4-32 g/ml. Neither colistin nor co-trimaxazole resistant BlaKPC-2 isolates.

TABLE 1: blaKPC-2 ppositive isolates' clinical features and antibiotic susceptibility testing results

SEX	AGE	SOURCE	MHT	MIC	IMI	MRO	CRO	CAZ	GEN	AMK
Male	**38**	**Urine.**	**++**	**5**	**5**	**R.**	**R.**	**R.**	**R.**	**S.**
Male	**65**	**Wound**	**++**	**4**	**32**	**R.**	**R.**	**S.**	**S.**	**S.**
Female	**12**	**Urine.**	**++**	**8**	**6**	**R.**	**S.**	**R.**	**R.**	**S.**
Male	**50**	**Wound**	**++**	**4**	**1**	**R.**	**R.**	**R.**	**R.**	**S.**
Female	**9**	**Blood**	**-**	**16**	**8**	**R.**	**S.**	**S.**	**R.**	**S.**
Male	**68**	**Wound.**	**++**	**4**	**0.5**	**R.**	**R.**	**R.**	**R.**	**R.**
Male	**47**	**Respiratory**	**++**	**32**	**8**	**R.**	**R.**	**S.**	**R.**	**S.**
Female	**46**	**Urine**	**++**	**8**	**5**	**R.**	**R.**	**.R.**	**R.**	**S.**

MHT: Modified. Hodge test, MIC: Minimum. Inhibitory Concentration, R: Resistant. S: Senstive
IMI: imipenem, M: male, F: female, GEN: gentamicin, MEM: meropenen

IV. CONCLUSION

Our institution found blaKPC-2 to be a carbapenem resistant K. pneumoniae pathway. Results demonstrated imipenem disc diffusion can quickly identify these isolates. These illnesses and their associated drug resistance demand infection control and antibiotic stewardship measures.

REFERENCES

[1]. Anand, T., Virmani, N., Kumar, S., Mohanty, A. K., Pavulraj, S., Bera, B. C., et al. (2020). Phage therapy for treatment of virulent klebsiella pneumoniae infection in a mouse model. *J Glob Antimicrob Resist.* 21, 34–41. doi: 10.1016/j.jgar.2019.09.018

[2]. Nyarko, R. O., Saha, P., Kumar, R., Kahwa, I., Boateng, E. A., Boateng, P. O., Christian, A., & Bertram, A. (2021). Role of Cytokines and Vaccines in Break through COVID 19 Infections. *Journal of Pharmaceutical Research International*, *33*(60B), 2544-2549. https://doi.org/10.9734/jpri/2021/v33i60B34912

[3]. Bae, J. Y., Jun, K. I., Kang, C. K., Song, K., Choe, P. G., Bang, J., et al. (2019). Efficacy of intranasal administration of the recombinant endolysin sal200 in a lethal murine staphylococcus aureus pneumonia model. *Antimicrob. Agents Ch.* 63:e2009-18. doi: 10.1128/AAC.02009-18

[4]. Briers, Y., Walmagh, M., Van Puyenbroeck, V., Cornelissen, A., Cenens, W., Aertsen, A., et al. (2014b). Engineered endolysin-based "artilysins" to combat multidrug-resistant gram-negative pathogens. *MBio* 5:e1379-14. doi: 10.1128/mBio.01379-14

[5]. Doss, J., Culbertson, K., Hahn, D., Camacho, J., and Barekzi, N. (2017). A review of phage therapy against bacterial pathogens of aquatic and terrestrial organisms. *Viruses* 9:50. doi: 10.3390/v9030050

[6]. Gutierrez, D., Garrido, V., Fernandez, L., Portilla, S., Rodriguez, A., Grillo, M. J., et al. (2020). Phage lytic protein lysrodi prevents staphylococcal mastitis in mice. *Front. Microbiol.* 11:7. doi: 10.3389/fmicb.2020.00007

[7]. KumarR., SahaP., KahwaI., BoatengE. A., BoatengP. O., & NyarkoR. O. (2022). Biological Mode of Action of Phospholipase A and the Signalling and Pro and Anti Inflammatory Cytokines: A Review. *Journal of Advances in Medicine and Medical Research*, *34*(9), 1-10. https://doi.org/10.9734/jammr/2022/v34i931342

[8]. Yang, H., Wang, M., Yu, J., and Wei, H. (2015). Antibacterial activity of a novel peptide-modified lysin against acinetobacter baumannii and pseudomonas aeruginosa. *Front. Microbiol.* 6:1471. doi: 10.3389/fmicb.2015.01471

[9]. Lim, J. A., Shin, H., Heu, S., and Ryu, S. (2014). Exogenous lytic activity of spn9cc endolysin against gram-negative bacteria. *J. Microbiol. Biotechnol.* 24, 803–811. doi: 10.4014/jmb.1403.03035

[10]. Grant, J. R., and Stothard, P. (2008). The cgview server: a comparative genomics tool for circular genomes. *Nucleic Acids Res.* 36, W181–W184. doi: 10.1093/nar/gkn179

[11]. Saha, P., Kumar, R., Nyarko, R. O., Kahwa, I., & Owusu, P. (2021). HERBAL SECONDARY METABOLITE FOR GASTRO-PROTECTIVE ULCER ACTIVITY WITH API STRUCTURES.

[12]. Dubey, A., Yadav, P., Verma, P., & Kumar, R. (2022). Investigation of Proapoptotic Potential of Ipomoea carnea Leaf Extract on Breast Cancer Cell Line. *Journal of Drug Delivery and Therapeutics*, *12*(1), 51-55Zurabov, F., and Zhilenkov, E. (2021).

[13]. Tomat, D., Migliore, L., Aquili, V., Quiberoni, A., and Balague, C. (2013). Phage biocontrol of enteropathogenic and Shiga toxin-producing escherichia coli in meat products. *Front. Cell. Infect. Microbiol.* 3:20. doi: 10.3389/fcimb.2013.00020

[14]. Awuchi, C. G., Amagwula, I. O., Priya, P., Kumar, R., Yezdani, U., & Khan, M. G. (2020). Aflatoxins in foods and feeds: A review on health implications, detection, and control. *Bull Environ Pharmacol Life Sci*, *9*(9), 149-155.

[15]. Kumar, S., Kumar, A., Kumar, R., Kumar, V., Kumar, N., & Tyagi, A. (2022). Phytochemical, Pharmacognostical and Pharmacological Activities of Carica papaya. *International Journal for Research in Applied Sciences and Biotechnology*, *9*(2), 310-315.