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Purposeful scientific research is a basic pillar for the advancement of Iraq

Molecular screening for presence mobile NDM-1gene in Imipenem-Resistant Escherichia coli strains isolated from Laboratories environment

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ABSTRACT

The production of the metallo-β-lactamase (MBL) enzyme, which generates the emergence of Carbapenem resistance in Gram-negative isolates.

Imipenem-resistant *Escherichia coli* isolates has been studied in this project, which will run from October 2020 to December 2020 in 3 microbiological laboratories chosen by the Ministry of Science and Technology.

One or more antibiotics were resistant in all isolates (100%) at all times. One hundred percent of the isolates had a profile of multi-drug resistance. All found to be resistant to ceftazidime, ampicillin, and ampicillin with clavulanic acid. The most resistance was detected against ampicillin.

Escherichia coli isolates that were imipenem-resistant were investigated. These were tested using phenotypic tests such as the double-combined disc diffusion test for the production of metallo—lactamase (MBL) (CDDT). The gene NDM-1 was molecularly confirmed using PCR.

33.3% (10/31) of the 30 Escherichia coli isolates were identified to generate NDM-1, and 63% (19/31) exhibited imipenem-resistant. Depending on the PCR technique and the combined disk test.

The importance of strengthening the biosafety program and policies in laboratories is highlighted by this study. Implement and revalidate protocols at regular intervals are most acceptable related biosafety and biosecurity. Contamination in microbiology laboratories can be totally eliminated, it can be managed to reduce both its frequency of occurrence and seriousness of its consequences by introducing good laboratory practice

Key words: *Escherichia coli* - antibiotic resistant – Carbapenem -NDM- molecular detection

Introduction

Antimicrobial resistance, particularly among harmful bacteria, has been developing since the invention of antibiotics. AMR has expanded over the past few years and now poses a threat on a worldwide scale. By 2050, it is anticipated that AMR would be responsible for 10 million fatalities annually due to an incomplete understanding of the components involved and the lack of strategies to counteract it (O'Neill J, *et al.*, 2019). Use of antimicrobial medications excessively and inappropriately, which might result in harmful bacteria developing resistance, is one of the key contributing elements to this problem. AMR, on the other hand, can spread across bacteria by horizontal gene transfer. Commensal bacteria are particularly significant in this situation (Singh AK, *et al.*, 2018)

Due to their stability even in the presence of ESBLs and Ampicillin, carbapenems like Imipenem, Meropenem, and Ertapenem are widely employed as last resort antibiotics against MDR Enterobacteriaceae clinical isolates (Sheu, *etal.*, 2019).

They quickly penetrate the Gram-negative bacterial cell wall through outer membrane proteins (porins) and target the bacterial penicillin-binding proteins (PBPs), which have a broad antimicrobial spectrum and include Gram-negative bacteria (Meletis G., 2016). In hospitals, it is applied to treat serious infections. (Ben Maamar S,*et al.*, 2020).

Currently, the most significant clinical issue with antibiotic resistance is the rise of carbapenemase, which is produced by Gram negative bacteria (Bialvae,*et al.*,2015).

However, carbapenem-resistant bacteria have been discovered. Carbapenem resistance (CR) mechanisms in Enterobacteriaceae are complex and largely caused by the creation of acquired metallo-lactamases (MBLs) (L. Dortet ,*et al.*, 2018)

The most important problems of researchers working with microbial cultures are Microbial contamination, it is undesired introduction of impurities like bacterial into microbiology laboratory ,and also may spread via physical means and biological means (Muhammad *et al.*, 2015) and also by airborne

microorganisms in which it is simple to enter, transfer, and maintain the necessary cells in culture. This study investigated the presence of the New Delhi metallo-β-lactamase-1 (NDM-1)-producing *Escherichia coli* in Laboratories environment (Muhammad *et al*., 2015).

The best that we can tell, no studies have been published on the rate of in Metallo—Lactamase in Imipenem-Resistant Escherichia coli isolates in laboratories. (Mahmoodi F, *et al*., 2020).

The purpose of this study was to identify MBL-producing E. coli strains and MBL genes in laboratory environments.

Materials and methods

Sample collection & processing

Sampling from environmental surface of microbiology laboratory (benches, tables, sink), using sterilized Swiffer pads, for each sampled area, ca. 100 cm2 was touched three to five times with the sterilized Swiffer pads to ensure that the entire area was sampled. Then put in the Bag sealed (Figure -1-) and added 50 ml peptone water, mixing well and transfer the growth to another selective medium (macConkey broth), incubated for 18-24 hr at 37C. positive results (change in colour of media) was inoculated onto blood agar , MacConkey agar and incubated 24-48 hr at 37C , then confirmed by doing Gram stain, catalase test, coagulase test.

Antibiotic susceptibility testing

Thirty *Escherichia coli* isolates were tested for resistance to twelve antibiotic agents, including: Amikacin, Cefotaxime, Ceftriaxone ,Ceftazidime, Aztreonam, Ciprofloxacin, Cefepime, Imipenem, Gentamicin , Piperacillin, Amoxicillin + clavulanic acid ،Ampicillin.

Phenotypic Detection of MBL Activity

Peptone water medium was employed to dilute an overnight culture of an *Escherichia coli* both clinical and environmental isolate to 10^5 CFU/mL to spreading it on a Mueller-Hinton (MH) agar plate. On the agar surface, twin IPM disks were placed close to one another, 4-5 cm apart(Thapa *et al*., 2017).

Next step was to add 5 ul of a 0.5 M EDTA solution onto one of the IPM disk. After 14 to 16 incubation hours at 37°C, the zones of inhibition from around IPM and IPM-EDTA disks were compared. A variation of 7 mm between both the zones of inhibition diameter of the IPM-EDTA disk and of the IPM only disk was regarded as a positive for the occurrence of MBLs (Panchal,*et al*., 2017).. The process was carried out twice to make sure

Genotyping Detection of MBL Activity

According to the manufacturer's instructions, the genomic DNA purification kit (Promega, USA) was used to extract the DNA from bacterial isolates. All isolates undergo molecular screening utilizing the PCR amplification technique to search for (NDM-1)-genes. This method makes use of primers (Alpha DNA, Canada).

(NDM-F-5′- GCAGCTTGTCGGCCATGCGGGC -3′,NDM-R-5′-GGTCGCGAAGCTGAGCACCGCAT-3′). PCR amplification was carried out in 50 ul reaction volume using Labnet ThermoCycler instrument (MultiGene OptiMax, USA).

The master mix was spiked with template DNA from one to two colonies of pure culture, following PCR protocol was used to find the NDM gene (Gonçalves,*et al*., 2017). After PCR products were electrophoresis on agarose gels with ethidium bromide, then were analysed under ultraviolet light.

Results and discussion

A total of fifty samples of environmental surface of microbiology laboratory (benches, tables, sink), tap water of laboratory were collected from 3 microbiological laboratories in ministry of science and technology and clinical samples were collected from private clinical laboratory, from October 2020 to December 2020, Specimens processed in this study were clinical (n=15), and environmental (n=35) (figure).

from 50 sample only 30 (60%) bacterial isolate was identifying as *Escherichia coli* obtained according to the morphological, cultural and biochemical characters (figure 2), Under a microscopic examination, isolated bacteria appeared as gram-negative rods that are not really spore-forming and were regarded to be E. coli. The hue green with a metallic shine (figure 2 (Quinn, *et al*., 2004).

The antibiotic resistance profiles were as follows.: All isolates (100%) were resistant to one or more than one antibiotic. Multi-drug resistant isolates profile was observed in (100%) isolates. All isolates were resistant to(Ampicillin, Amoxicillin+ clavulanic acid, ceftazidime), The most resistance was observed against Gentamicin(76.7.2%), followed by Amikacin and Aztreonam (66.6%) and Imepenem (63%), Cefotriaxime (56.6), Ciprofloxacin (53.3%), Cefotriaxon (46.6) (figure 3).

Escherichia coli had the greatest percentage of multi-drug resistant in our study, which is analogous to multi-drug resistant reported by (Strateva *et al*., 2007), However, other research projects performed out in Nepal indicated somewhat more multi-drug resistant incidences (Mishra, *et al*., 2014). it is widespread in many nations, the World Health Organization reports. However, there is no precise estimate of the scope of the issue or the economic damages brought on by AMR (WHO, 2014), However, precise information on AMR isolates is lacking. in the environmental and clinical samples of research laboratories in our country. So, in this research, the occurrence of AMR was assessed. in microbiological laboratories were selected in ministry of science and technology

(Fig -1-) Bag sealed

Fig 2: Escherichia coli colonies appeared as metallic sheen in EMB medium

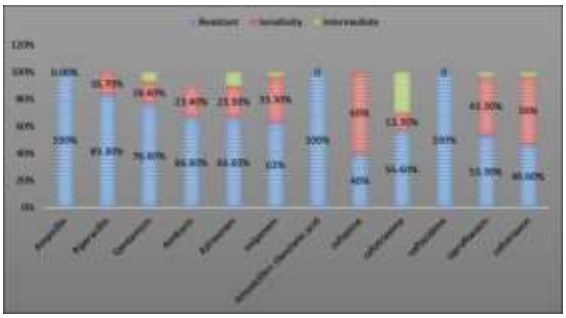


Fig 3: Antibiotics Sensitivity Tests

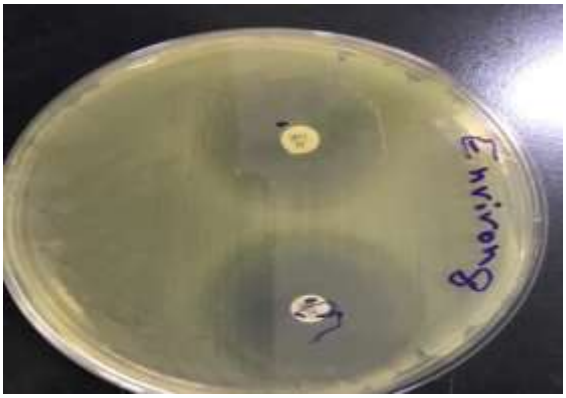


Fig 4: Combined Disk Test

There are currently no guidelines for the identification of these enzymes, despite the fact that there are a number of screening techniques used and advised for the detection of MBL production. In this research, however, EDTA soaked IMP disc indicated improved sensitivity in the detection of MBL, which is similar to the results of other researchers (Jesudason, et al., 2005: Lee, *et al*., 2003).

One of the most important considerations is in the detection of bacteria that carry the carbapenem-resistant gene (Gautama, *et al*., 2020).

The best method for finding isolates that produce Metallo-β-lactamase is typically a PCR-based approach. However, as PCR primers are typically intended to identify a particular gene type, testing every suspected isolate using PCR-based methods would be costly and unacceptable from a budgetary standpoint. In order to get around this issue, we adopted the imipenem-EDTA combination disk approach for phenotypic identification, which has a sensitivity and specificity of 100 and 98%, respectively, and is also reasonably priced (Lee K, *et al*., 2004).

Metallo-β-lactamase was first identified in the middle of the 1960s as a zinc-dependent enzyme in Bacillus cereus. A few years later, enterobacteriacaea were revealed to contain imipenem hydrolyzing metallo. These enzymes were first only found in a single clinical isolate and were generated by chromosomal genes. Escherichia coli and other bacteria were also found to produce Metallo-β-lactamase (Ejikeugwu C,, *et al*., 2021).

NDM-1 has been found both in the bacterial chromosome and plasmid. In addition to genes imparting resistance to beta-lactams, plasmids bearing the bla NDM-1 gene also carry several other genes for aminoglycoside, macrolide, and sulfamethoxazole resistance. As a result, NDM-producing strains are resistant to almost all classes of drugs that are currently on the market. The presence of an NDM producer in a hospital setting is concerning because the plasmids present in such strains are easily transferrable and have the potential for extensive horizontal transfer. People may carry these strains in their bodies without showing any symptoms. There are no common, standardized phenotypic tests to identify it (Rolain,*et al*., 2010).

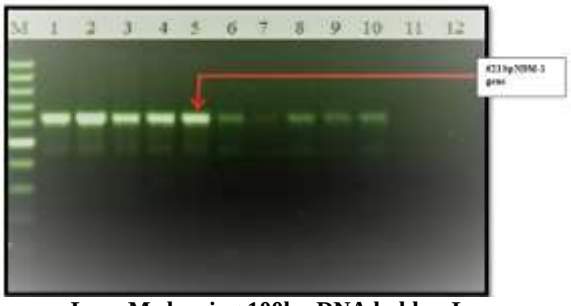
Our study's results showed a higher incidence of multi-drug resistant and Metallo—lactamase -positive *Escherichia. coli*, which have been linked globally to negative clinical outcomes such a higher risk of morbidity and mortality. The findings of the study show that the emergence of metallo-beta-lactamase producers poses a severe therapeutic and epidemiological risk. As a result, it is crucial to identify Metallo—lactamase producers in order to treat these illnesses effectively and to stop the spread of resistance.

Since significant multi-drug resistant strains were detected in nearly half of the Metallo—lactamase producers, the most recent findings are concerning. Only a few researches had looked into the familiarity with and adherence of laboratory employees to

common biosafety precautions. Low-resource areas are mostly where biosafety is compromised. This study showed that biosafety is disregarded in research laboratories, which highlights the need to develop biosafety programs and policies, especially in such facilities. All laboratory personnel should receive written biosafety procedures, biosafety training, and monitoring for compliance with established laboratory safety practices. International standards should be followed, including the application of suitable microbiological techniques, adequate facilities, protective barriers, and specialized training and instruction for laboratory staff. Moreover, laboratory facilities must be built and designed properly.

Conclusion

Nineteen *Escherichia coli* isolates resistant to IMP (10 µg) using disc diffusion profile were obtained from both environmental and clinical samples, depending on Combined Disk Test and PCR method, 12(40 %) isolates were product New Delhi metallo-β-lactamase-1 (NDM-1) in clinical and environmental samples, the zone of inhibition surrounding both IMP discs was measured and compared. It is indicative of Metallo-β-lactamase synthesis when the inhibition zone size increases for at least seven millimeter around the IMP-EDTA disc,this study revealed that laboratory employees had fair to inadequate biosafety information, practices, and commitments, as seen by the low percentage of laboratory workers who got a biosafety manual and training.



Lane M showing 100bp DNA ladder, Lane 1,2,3,4,5,6,7,8,9 and 10 showing 621 bp amplified PCR product of NDM-1 gene, while Lane 11 showing no amplification. Lane 12 showing negative control.

References

Ben Maamar, S., Glawe, A. J., Brown, T. K., Hellgeth, N., Hu, J., Wang, J. P., ... & Hartmann, E. M. (2020). Mobilizable antibiotic resistance genes are present in dust microbial communities. *PLoS pathogens*, *16*(1), e1008211.

Bialvaei, A. Z., Kafil, H. S., Asgharzadeh, M., Yousef Memar, M, & Yousefi, M. (2016). Current methods for the identification of carbapenemases. *Journal of Chemotherapy*, *28*(1), 1-19.

Dortet, L., Bernabeu, S., Gonzalez, C., & Naas, T. (2018). Evaluation of the Carbapenem Detection Set™ for the detection and characterization of carbapenemase-producing Enterobacteriaceae. *Diagnostic Microbiology and Infectious Disease*, *91*(3), 220-225.

Ejikeugwu, C., Nworie, O., Saki, M., Al-Dahmoshi, H. O., Al-Khafaji, N. S., Ezeador, C., ... & Adikwu, M. U. (2021). Metallo-β-lactamase and AmpC genes in Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa isolates from abattoir and poultry origin in Nigeria. *BMC microbiology*, *21*(1), 1-9.

Gautam, S., Bhattarai, N. R., Rai, K., Poudyal, A., & Khanal, B. (2020). Detection of bla NDM-1 encoding imepenemase among the imipenem-resistant gram-negative bacilli isolated from various clinical samples at a tertiary care hospital of eastern Nepal: a descriptive cross-sectional study. *International Journal of Microbiology*, *2020*.

Gonçalves, G. B., Furlan, J. P. R., Vespero, E. C., Pelisson, M., Stehling, E. G., & Pitondo-Silva, A. (2017). Spread of multidrug-resistant high-risk Klebsiella pneumoniae clones in a tertiary hospital from southern Brazil. *Infection, Genetics and Evolution*, *56*, 1-7.

Jesudason, M. V., Kandathil, A. J., & Balaji, V. (2005). Comparison of two methods to detect carbapenemase & metallo-beta-lactamase production in clinical isolates. *Indian Journal of Medical Research*, *121*(6), 780.

Lee, K., Ha, G. Y., Shin, B. M., Kim, J. J., Kang, J. O., Jang, S. J., ... & Korean Nationwide Surveillance of Antimicrobial Resistance (KONSAR) group. (2004). Metallo-β-lactamase-producing Gram-negative bacilli in Korean Nationwide Surveillance of Antimicrobial Resistance group hospitals in 2003: continued prevalence of VIM-producing Pseudomonas spp. and increase of IMP-producing Acinetobacter spp. *Diagnostic microbiology and infectious disease*, *50*(1), 51-58.

Lee, K., Lim, Y. S., Yong, D., Yum, J. H., & Chong, Y. (2003). Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of Pseudomonas spp. and Acinetobacter spp. *Journal of clinical microbiology*, *41*(10), 4623-4629.

Mahmoodi, F., Rezatofighi, S. E., & Akhoond, M. R. (2020). Antimicrobial resistance and metallo-beta-lactamase producing among commensal Escherichia coli isolates from healthy children of Khuzestan and Fars provinces; Iran. *BMC microbiology*, *20*(1), 1-11.

Meletis, G. (2016). Carbapenem resistance: overview of the problem and future perspectives. *Therapeutic advances in infectious disease*, *3*(1), 15-21.

Mishra, S. K., Awal, B. K., Kattel, H. P., Acharya, J., Gyawali, P., Bhatt, C. P., ... & Pokhrel, B. M. (2014). Drug resistant bacteria are growing menace in a

University Hospital in Nepal. *Am J Epidemiol Infect Dis*, *2*(1), 19-23.

Muhammad Ghayoor, Abdul Qadoos, Sulaiman Bahadar, Azam Hayat, Muhammad Daud, Adil Hassan, Fahad Ali, Aurang Zeb, Khalil Ur Rahman, Abdul Wahab, Zeenat Fatima Khattak and Baharullahkhattak (2015). Isolation and Identification of Common Contaminants Bacteria from Working Area in Microbiology Laboratory. Journal of Bio-Molecular Sciences (JBMS) 3(2): 74-78.

Panchal, C. A., Oza, S. S., & Mehta, S. J. (2017). Comparison of four phenotypic methods for detection of metallo-β-lactamase-producing Gram-negative bacteria in rural teaching hospital. *Journal of laboratory physicians*, *9*(02), 081-083.

Quinn PJ, Carter ME, Markey B, Carter GR (2004) Clinical Veterinary microbiology. 6th ed. Mosby an imp. Wolf, London. 66 – 85.

Rolain, J. M., Parola, P., & Cornaglia, G. (2010). New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia?. *Clinical microbiology and infection*, *16*(12), 1699-1701.

Sheu C-C, Chang Y-T, Lin S-Y, Chen Y-H, Hsueh P-R. (2019)Infections caused by carbapenem-resistant enterobacteriaceae: an update on therapeutic options. *Front Microbiol*. 10:80.2019.

Singh, A. K., Das, S., Singh, S., Gajamer, V. R., Pradhan, N., Lepcha, Y. D., & Tiwari, H. K. (2018). Prevalence of antibiotic resistance in commensal Escherichia coli among the children in rural hill communities of Northeast India. *PloS one*, *13*(6), e0199179.

Strateva, T., Ouzounova-Raykova, V., Markova, B., Todorova, A., Marteva-Proevska, Y., & Mitov, I. (2007). Problematic clinical isolates of Pseudomonas aeruginosa from the university hospitals in Sofia, Bulgaria: current status of antimicrobial resistance and prevailing resistance mechanisms. *Journal of medical microbiology*, *56*(7), 956-963.

Thapa, P., Bhandari, D., Shrestha, D., Parajuli, H., Chaudhary, P., Amatya, J., & Amatya, R. (2017). A hospital based surveillance of metallo-beta-lactamase producing gram negative bacteria in Nepal by imipenem-EDTA disk method. *BMC research notes*, *10*(1), 1-6.

World Health Organization. (2014). *Antimicrobial resistance: global report on surveillance*. World Health Organization.

Zhao, Y., Cocerva, T., Cox, S., Tardif, S., Su, J. Q., Zhu, Y. G., & Brandt, K. K. (2019). Evidence for co-selection of antibiotic resistance genes and mobile genetic elements in metal polluted urban soils. *Science of the Total Environment*, *656*, 512-520.

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