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DETECTION OF CO-EXISTENCE OF CARBAPENEMASE GENES USING MULTIPLEX PCR AMONG GRAM NEGATIVE BACTERIA ISOLATED FROM TERTIARY CARE HOSPITAL IN HYDERABAD

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Abstract:

Introduction: The emergence of carbapenem resistant organisms has been causing a grave challenge to the physicians and healthcare workers to treat the infections. Several carbapenemase genes have been reported to be involved in the development of carbapenem drug resistance. In the present study we intend to evaluate the role and presence of carbapenemase genes KPC, VIM and OXA-48 like through multiplex PCR in different gram negative bacteria.

Materials and Methods: A total of 100 bacterial isolates from the clinical samples urine -60, endotracheal secretions -18, blood culture -11, pus -8 and sputum-3. The gram negative bacteria isolates included *Escherichia coli* -53, *Klebsiella pneumoniae-30, Pseudomonas aeruginosa- 13 and Acinetobacter Sp.-4*. The organisms isolated were subjected to Kirby Bauer Antibiotic sensitivity test. The DNA extraction has been performed using bacterial DNA extraction kit (Quiagen QIAmp kit) as per the instructions of the manufacturer. The multiplex PCR has been used for the detection of *KPC, VIM* and *OXA-48* like.

Results: The carbapenem drug resistance has been noticed in 35% of cases. The PCR has been detected VIM in 22% of isolates OXA - 48 in 20 % of isolates and KPC 13% of isolates. In 5% of cases in which the routine antibiotic sensitivity cases were showing sensitive to meropenem and imipenem has shown the presence of KPC carbapenem resistant genes.

Conclusion: Our study has shown the coexistence of multiple genes in a single bacteria pointing out that different carbapenmases enzymes are utilized by the bacteria to inactive the carbapenem drugs. The infection control practices and antibiotic stewardship will ensure the prevention of spread of carbapenem resistance.

Introduction

The most effective antibiotics used worldwide for the treatment of infections are the β-lactam drugs. These drugs include classes of penicillins**,** cephalosporins, monobactams and carbapenem. These antibiotics share a common structure of presence of β-lactam rings and the common action of inactivating the penicillin binding protein, which plays an important role in the cell wall formation of the bacteria. [1] In recent years carbapenems are playing an important role in limiting the infections caused by both gram positive and gram negative bacteria due to the presence of carbapenem along with the β-lactam ring, which confers stability against the action of β-lactamase enzymes and extended β-lactamases (ESBL). [2] Carbapenems are the drug of choice of β-lactam resistant organisms.

The emergence of carbapenem resistant organisms has been noticed especially in gram negative bacteria, causing a grave challenge to the physicians and healthcare workers to treat the infections. Bacteria evolved several mechanisms to overcome the action of carbapenem drugs including reduction in the permeability of their outer membrane through the down regulation of the oprD through deletion mutations. The presence of efflux pump with the ability to efflux the carbapenem such as meropenem, and has been associated with multidrug resistant organisms (MDR).[3] Enzyme mediated resistance to carbapenem is through production of carbapenmases. These enzymes have ability to inactive carbapenem along with other β-lactam antibiotics. The carbapenemase enzymes such as KPC, [4] GES/IBC belongs to group A, MBLs, IMP,VIM [5] belong to group B and several OXA-type [6] belong to group D play an important role in hydrolysing the carbapenems. Enzyme mediated resistance is more important clinically as they have the ability for horizontal transfer through plasmids or transposons.

Studies have shown the presence and prevalence of the carbapenem resistant genes worldwide. Wang and co-workers demonstrated the high prevalence of carbapenem resistant genes in Western China. [7] Carbapenem resistance mechanisms, prevalence and molecular epidemiology has been surveyed in Latin America and found that presence of high carbapenem resistance exists in several countries of Latin America and predominantly due to the presence of oxacillinases OXA-23, OXA-58 and (in Brazil) OXA-143 genes. [8]

High prevalence of carbapenem resistance has been noted in India, a study conducted by Sekar et al has found the

presence of carbapenemase gene NDM-1 in the *Klebsiella* sps isolated from paediatric bacteraemia. [9] Increased rise of carbapenem resistance has been noticed in the due to presence of carbapenem resistant genes located on the plasmids and ability for horizontal transfer. [10]

Our study has been carried as we found high carbapenem drug resistance in our laboratory. We intend to evaluate the role and presence of carbapenemase genes KPC, VIM and OXA-48 like through multiplex PCR in different gram negative bacteria isolated in the Mmicrobiology department, Kamineni Academy of Medical Sciences and Research Centre, Hyderabad.

Material and Methods

Bacterial isolates and antibiotic susceptibility testing

The study was conducted in the Department of Microbiology, Kamineni Academy of Medical Sciences and Research Centre from January 2018 to August 2018. A total of 100 bacterial isolates from the clinical samples urine -60, endotracheal secretions -18, blood culture -11, pus -8 and sputum-3. The organisms isolated were subjected to Kirby Bauer Antibiotic sensitivity test for the antibiotics including Ampicillin (10µg), Amoxicillin Clavulanate (20/10 µg), Gentamicin (10µg), Amikacin (30 µg) , Netilmicin (30 µg), Tetracycline (30 µg), ciprofloxacin (5 µg), Trimethoprim Sulfamthoxazole, Piperacillin tazobactam (100/10 µg), cefazolin (30 µg), cefipime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), cefoperazone (75 µg), cefuroxime (30 µg), Ertapenem (10 µg), Imipenem (10 µg), Meropenem (10 µg), for urine isolates Norfloxacin (10 µg) and Nitrofurantoin (300 µg).

DNA Extraction and PCR

The DNA extraction has been performed using bacterial DNA extraction kit (Quiagen QIAmp kit) as per the instructions of the manufacturer. Quality of the DNA was assessed using 1.0% agarose gel electrophoresis (AGE) and the isolated DNA was stored at -20° C, until use. PCR was carried out in a total volume of 50 µl PCR reaction mixture, using specific primers. The multiplex PCR has been used for the detection of *KPC, VIM* and *OXA-48* like. According to the instructions of Mlynarcik et al [11]. The PCR has been carried out in PCR in(Takara,).Initial denaturation was carried out for 5 minutes at 95°C, followed by 35 cycles of denaturation at 95° C for 1 minute, annealing at 56° C for 1 minute, extension at 72° C for 1 minute and a final extension at 72° C for 10 minutes. The amplicons obtained were of the expected size KPC-340bp, VIM-247bp and OXA-48 585 bp, has been evaluated and examined in 1.5% agarose gel electrophoresis using 100bp ladder in gel doc (Aga MIDI transilluminator).

Results

The patients including females-48 ,males-52 with a mean age of 51.1 years (4-77yrs).The gram negative bacteria isolated are 100 isolates included *Escherichia coli* -53, *Klebsiella pneumoniae-30, Pseudomonas aeruginosa- 13 and Acinetobacter Sp.-4*. The resistance pattern shows high drug resistance to ciprofloxacin (65%), ofloxacin (60%), gentamicin (69%), netilimicin (77%), Ceftazidime (73%), Ceftraixone (66%), Cefotaxime (65%), Imipenem (35%) and meropenem (35 %) of cases. The PCR has been detected VIM in 22% of isolates OXA - 48 in 20 % of isolates and KPC 13% of isolates. In 5% of cases in which the routine antibiotic sensitivity cases were showing sensitive to Meropenem and Imipenem has shown the presence of KPC carbapenem resistant gene (figure 1). The co expression of genes has been noticed including VIM & OXA-48 in 2%,VIM & KPC in 4% and OXA-48 & KPC in 4% and all genes expressed in 3% of cases .The *E coli* has shown presence of carbapenem resistant genes in 23 % of cases, *Klebsiella spp* & *Pseudomonas aeruginosa* each 7% of cases.

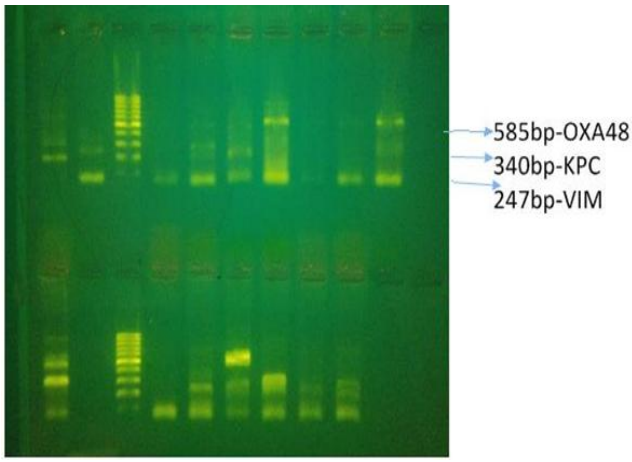

Figure 1: Detection of OXA48, KPC and VIM gene in multidrug resistant gram negative bacteria

Discussion

We have evaluated the reliable, rapid multiplex PCR for the detection of VIM, OXA-48 and KPC genes in gram negative bacteria isolated in our department. We found highest expression of VIM in 22% of cases in accordance with the study done by Elappan et al which found presence of VIM gene in 23% of cases and found presence of coexistence of multiple mode of drug resistance mechanisms. [12] We also found the existence of OXA-48 in 20% of cases similar to Sahin et al [13] and KPC gene in 13% of cases which echoed the similar results in studies conducted by Iradia et al [14] and Priyadarshini from India.[15] Through the application of multiplex PCR we have detected the coexistence of multiple carbapenem resistant genes in gram negative bacteria VIM & OXA-48 in 2%,VIM & KPC in 4% and OXA-48 & KPC in 4% and all genes expressed in 3%

of cases. Previous studies also pointed out the coexistence of multiple genes in a single bacteria pointing out that different carbapenmases enzymes are utilized by the bacteria to inactive the carbapenem drugs. [12, 16]

In our study we have found that in 5% of cases in which the gram negative bacteria are sensitive to imipenem and carbapenem but have shown the presence of KPC gene similar to the study conducted by Weisengberg et al [17] which confirmed that out of the 28 cases of KPC producing Klebsiella pneumoniae 46% of cases has shown susceptible to imipenem. It indicates KPC producing bacteria may be misidentified through the use of routine antibiotic susceptibility testing, and resistance to ertapenem may correctly identify the KPC producing bacteria.

To conclude the prevalence of the carbapenem genes is highly prevalent and it is spreading at a rapid pace due to horizontal transfer. The evaluation for the presence of multiple carbapenem genes through the application of multiplex PCR helps in early intervention. Stringent practices of infection control and antibiotic stewardship will ensure the prevention of spread of carbapenem resistance in health care centers and hospitals.

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