Review Article

Carbapenem Resistance Of *Pseudomonas Aeruginosa*: A Review

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ABSTRACT

Carbapenem resistance towards the gram negative microorganisms especially *Pseudomonas aeruginosa* is alarming and on-going public health problem all over the world. It may be intrinsic or acquired through transcription of genes among microorganisms. These genes are spreading rapidly and are responsible for serious out-breaks. So selection of antibiotics is limited to treat these resistant cases. Resistant genes are commonly extended in Europe, Asia (Turkey, India, China, Pakistan and so on) and South America. In Pakistan carbapenem resistance in Pseudomonas aeruginosa isolates is increasing among hospitalized patients. It shows a progressive trend in multidrug resistance (even towards last resort drug carbapenem). In this article, we offer an in-depth review of carbapenem resistant *Pseudomonas aeruginosa*. This will facilitate the readers to take effective measures in order to control infection and appropriate use of antibiotics.

Keywords: appropriate antibiotics, Carbapenem resistant, transcription of genes.

INTRODUCTION:

Pseudomonas aeruginosa is opportunistic and non fastidious pathogen. This bacterium is a threat in particular to those hospitalized patients who are dependent on devices as ventilators, blood catheters, urinary catheters, i/v (intra vascular) catheters etc.[1]It leads to pneumonia, meningitis, skin infections, urinary tract infections, endophthalmitis and malignant otitis externa.[2] Different anti-pseudomonal drugs are available but it exhibits multidrug resistance. It has natural aptitude to adopt new ways to resist treatment. So carbapenem is a good choice but many surveillance studies conducted in USA and Europe have shown the increasing prevalence of CRPA (Carbapenem Resistant Pseudomonas Aeruginosa).[3, 4] Beta- lactamase and different genes are responsible for resistance.[5, 6, 7] Annually, worldwide antimicrobial resistance was projected to cost over $ 105 billion dollars.[8] In Pakistan,isolates of Pseudomonas aeruginosa are also detected to have a progressive tendency towards carbapenem resistance.[9] WHO publishes an antibiotic resistant list in which *P. aeruginosa* exhibits carbapenem resisttance is considered as critical pathogens.

METHODOLOGY:

Google and Google scholar search engine were employed with numerous key words and idioms to search articles related to antibiotic resistance epidemiology in the Gram negative rod shaped bacilli *Pseudomonas aeruginosa.* Articles were selected from 2013 to 2018 for write up of this review. Key words such as carbapenem resistance, *Pseudomonas aeruginosa* modified Hodge test and genes detection were used. A total of 200 articles included 5 review articles and 195 original articles. Among 200 articles 40 were short listed on the basis of correlation with my work. A major content of this article is based on genotype detection of antibiotic resistance by Molecular detection of genes that code for enzymes that cause resistance towards traditionally used antibiotics.

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LITERATURE REVIEW:

All over the World carbapenem starts to show its resistance especially hospitalized patients.[10]

Pathogenesis consists of different virulence factors like endotoxin, exotoxin, enzymes, adhesions, biofilms and pigments etc. Endotoxin A is responsible for tissue necrosis, it restrains protein synthesis through ADP-ribosylation of elongation factor-2. Different enzymes eg elastase and proteinases (zinc-metalloprotease and metalloendopeptidases) that make easy incursion into blood-stream. Adhesion through pili can kick off the biofilm formation and phagocytosis. Pigments especially pyocynin can disrupt the movement of cilia and facilitate the secretion accumulation.

LPS (lipopoly-saccharides) are recognasized by TLR4-MD2-CD14 which are located on macrophages and dendritic cells, so these take part in inflammation. Flagellum also plays in adhesion,invasion, biofilm formation and mediation of inflammation. Type IV pili binds to glycosphingolipid located on host epithelial cell-membranes.this facilitates the internalization of *P. aeruginosa.* Type IV pili contains pilin protein and indulges in inflammation.

Different drugs are in practice to control *pseudomonal* drugs eg β lactum antibiotics (penicillin, cephalosporine and carbapenem), fluoroquinolone and aminoglycosides. P. aeruginosa is defiant to multiple antibiotics because of unnecessary and inadequate use of these antibiotics.

CARBAPENEM ANTIMICROBIALS:

Carbapenems are β-lactam antibiotics. It binds to pencillin-binding proteins (PBP) and hinder the production of cell wall of micro organism.It is effective against both gram positive and gram negative bacteria[11,12]

Carbapenems were produced from thienamycin that is derivative of streptomyces cattleya. Carbapenems are analogous of pencillin but the sulfur atom in position 1 of the structure has been substituted with a carbon atom. Imipenem is the other antibiotic in this group which was introduced clinically in the United States of America in the year 1985.[13]

Carbapenems cannot move through bacterial cell wall. It pierces through porins. While crossing through the periplasmic space, carbapenems undyingly acylate the PBP (pencilline binding proteins). Carbapenem acts as inhibitors of the enzymes of PBP. At the end cell death of microorganism occurs due to osmotic pressure.[14]

Carbapenems are effective as Empiric monotherapy for ventilator associated pneumonia, intraabdominal infections and infections in cancer patients.[15] It is contraindicated in those who are hypersensitive because of increased risk of seizure.[16] Carbapenem can interact with live typhoid vaccine and probenecid. Different mechanisms are involved in carbapenem resistance like:

a. Loss of outer-membrane porins the upregulation of an efflux pump:

Pseudomonas aeruginosa exhibits resistance to carbapenem due to impermeability through cell-membrane. This impermeability is mediated by MexA-MexB-OprM. Gram negative organism's membrane is naturally designed with pores of Opr-M. MexB protein facilitates the exit portal. This pump comes across impermeability of drugs by upregulation of Mex A-MexB-OprM.This upregulation crop up as a result of nalB mutation.On the other hand Pseudomonas aeruginosa is also set up to lack Opr D proteins. These OprD pores allow the entrance of carbapenems. Whenever these pores are lost, carbapenems have to face the challenges of resistance.

b. Enzymes β-lactamases

β-lactamases are the major reason of microbial resistance to β-lactam medicine. There are four molecular classes of β-lactamases A, B, C and D according to Ambler classification. Three classes out of four posses serine amino acid at active site. These classes are A, C and D.

Class A β-lactamases are the most assorted and widely allocated class of the β-lactamases.[18] This class belongs to different enzymes which are chromosomally encoded, for instance NmcA (not metalloenzyme carbapenemases A), SME (Serratia marcescens enzyme), IMI-1 (Imipenem-hydrolyzing β-lactamases), SFC-1(Serratia fonticola carbapenemases-1). But plasmid encoded enzymes are KPC (kleibsiella pneumoniae carbapenemases) and GES (Guiana extended spectrum). Carbapenemases A is monomeric enzyme consisting of 265-269 amino acids. These enzymes inactivate β-lactams by hydrolysis before it reaches the PBP targets. In serine β-lactamases hydroxyl group breaks β-lactam ring. But in case of class B requires Zn^{+2} to facilitate the process of hydrolysis. KPC had spread all over the World especially Asia, NorthAmerican, European countries and Africa.[19]

Class B carbapenemases consists of enzymes New Delhi metallo- β-lactamase 1(NDM-1), Imipenem-resistant pseudomonas (IMP)-type carbapenemases, VIM (Verona integron-encoded metallo-β-lactamase), GIM (German imipenemase) and SIM (Seoul imipenemase). The NDM-7 carbapenemase has been recognized in 2008 in Escherichia coli in France.NDM-1 producing P. aeruginosa isolates are major intimidation to human beings.[20] . These enzymes were detected in pseudomonas, Acinetobacter and Enterobactericeae.[21]

Class D carbapenemases carbapenemases are OXA (Oxacillinase) enzymes. These enzymes are stumbled on P. aeruginosa and Acinetobacter bauminnii. OXA was first discovered in 1985 in Edinburgh, Scotland, by Pton et al. OXA type carbapenemases are majorly discrete in *P aeruginosa* and *A. baumannii.* Oxacillinase hydrolyses the isoxazolylpencillin oxacillin. Presently 121 variants of class D β-lactamases have been well-known on the basis of protein and 45 of them reveal carbapenem-hydrolysing activities.[22]

c. Intrinsic resistance

Pseudomonas aeruginosa acquires high intrinsic resistance. This resistance towards antibiotic inflicts financial burden and patient health.[23] intrinsic resistance occurs as a result of genes from naturally existing bacteria to clinical pathogen.

THREATS FOR ACQUIREMENT OF CARBAPENEM RESISTANCE

Resistant isolates of *Pseudomonas aeruginosa* can cause infections in immune-supressed conditions (neutropenia), elderly patients, hospital acquired infections (mechanical ventilation and organ transplantation), previous exposure to antibiotics, inadequate use of antibiotics and unnecessary use of antibiotics. In order to over-come the problems of antibiotic resistance, nonjudicious use of antibiotics in animals and plants should be avoided. Carbapenem resistance is common in developing countries in sub-saharan Africa.[24]

First report of carbapenem resistance from an aeromonas hydrophila isolate was observed in 1980 in Japan. Consecutively followed in London (1982) from Serratia marcescens (SME-1), IMI-1 from Enterobacter cloacae in Calfornia (1984) and NMC-A from Enterobacter cloacae in France (1990). Carbapenem resistant KPC was firstly recognized in 1996 in United States. Out breaks of KPC

were existing in Israel, Greece, Colombia, Canada, Australia and New-zealand.[25]From 2000-2010, infection rates has boosted from 1% to 12% in United States.[26] KPC gene positive *Pseudomonas aeruginosa* had found majorly from 2009-2012, 231 KPC- positive strains in 2010, 368 in 2011 and 293 in 2012 were reported[27] Class D carbapenemases are recently classified under four sub-groups, sub-group-1 is made up of OXA-23, OXA-27 and OXA-49, sub-group-2 is composed of OXA-24, OXA-25, OXA-26, OXA-40, sub group 3 is made up of OXA-51 and sub group 4 is made up of OXA-58. In Turkey, carbapenemase resistance is at high rate and resistance is screened for IMP, VIM, OXA-23, OXA-27, OXA-49, OXA-25, OXA-26, OXA-40 andOXA-48. The bla OXA-48 gene in *pseudomonas aeruginosa* isolates were discovered from December 2015 to January 2017 in Khartoum state (Sudan).[29] OXA-48 producing *Klebsiella pneumoniae* is recently monitored in France, Belgium, Israel, Russia and the Netherland and exhibit resistance to carbapenem. The bla OXA-48 gene has been recognized as insertion sequenceIS1999 in *Klebsiella pneumonia*. The bla_{OXA-48} was part of transposon (Tn1999) was practical. Now Tn1999 has been discovered in an E. coli in Italy.[30] .But now OXA-48 producing isolates have been documented in Lebanon, Sultanate of Oman, Saudi Arabia and Kwait. Resistance to colistin in *Pseudomonas aeruginosa* is uncommon but has been occurred.[31] Resistance to all antibiotics except the polymyxin is now common in numerous hospitals. Daplano et al explained outburst of panresistant *Pseudomonas aeruginosa* in an intensive care unit in Belgium. In Japan, surveillance for multi-drug-resistant *Psedomonas aeruginosa* described the metallo-β-lactamase gene bla_{SMP-1} involved in resistance from 2004 to 2006.[32]

PHENOTYPIC AND GENOTYPIC METHODOLOGY

It can be detected by disc diffusion, MICs, selective agar, modified Hodge test, synergy test, spectrometry, genome sequencing and molecular methods.

Phenotypic detection

First of all *pseudomonas aeruginosa* is detected by gram staining from different samples as gram-negative organisms. The organisms can grow on blood agar and MacConkey agar. On blood agar, colonies are grey and irregular as fig no 1A.

MacConkey is used to differentiate fermenters from non fermenters, as *Pseudomonas aeruginosa* is non fermenters as per fig 1B. *Pseudomonas aeruginosa* can also be detected by biochemical test eg oxidase test as per fig 1C. It turns purple within few seconds. For disc diffusion, organism is inoculated on Muller-Hinton Agar.[33] Then organism is incubated overnight after placing antibiotic discs. Next day inhibitory zone around antibiotic disc is measured according to CLSI.[34]

The modified Hodge Test (MHT) is inexpensive and practicable method. It is used for carbapenemase detection phenotypically and recommened by CLSI.[35]

Break points for carbapenem is revised every year according to CLSI and EUCAST.[36,37]Most Eurpean, Asian and African countries use EUCAST and CLSI as guidelines to detect carbapenemase producing isolates. Whenever these

Fig 1A.

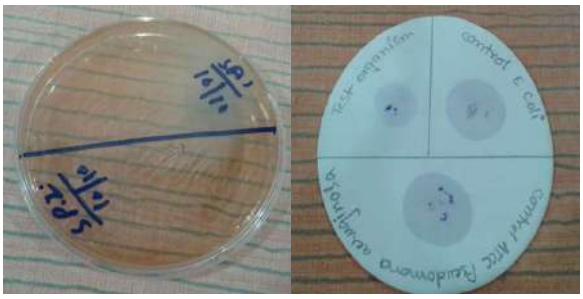

Fig 1B. Fig 1C

Antimicrobial Agents	Disk content	Zone diameter Break points			MIC Break points			Comments
		(Sens)	(Inter)	(Resist)	(Sens)	(Inter)	(Resist)	
Doripenem	10µg	>19mm	16-18mm	<15mm	<2mm	4mm	>8mm	Based on dosage regime of 500mg every 8hrs
Imipenem	10µg	>19mm	16-18mm	<15mm	<2mm	4mm	>8mm	1gm for 1hr and 500mg every 6hrs
Meropenem	10µg	>19mm	16-18mm	<15mm	<2mm	4mm	>8mm	1gm every 8hrs

Zone Break Points And Mic Break Points As Per CLSI

Table 1: As per CLSI

recommendations are delayed, clinicians and researchers come across problems. Modified Hodge Test is time consuming and unable to detect MBL, but 100% sensitive for class A and D.[38,39]

Genotype based technique

Molecular techniques are the most reliable methods for confirmation of carbapenemase production and resistance. Colonies of *pseudomonas aeruginosa* are detected by polymerase chain reaction within 4-6 hours with specific sensitivity and specificity. Wang et al informed a real time PCR with 100% sensitivity and specificity. Plasmid located genes like NDM, VIM, IMP and class D serine carbapenemase OXA-48 need to be identified on molecular basis. This procedure will improve the detection of unidentified genes and variants of these genes. A molecular technique consists of different methods like multilocus sequence typing, multilocus enzyme electrophoresis and DNA finger printing methods. OXA-48 is carbapenem-hydrolyzing oxacillinase and mostly ubiquitous carbapenemase in Europe and Middle East. Several variants of OXA-48, but most frequent OXA-48 like carbapenemases, have been documented which change from OXA-48 on basis of amino acids e.g, OXA-162, OXA-163, OXA-181 and OXA-204.[40]

It is confirmatory test for detection of carbapenem resistance. It is fast and reliable methods helping diagnosis and treatment.[41] Molecular methods can detect and differentiate carbapenemase consisting of NDM, KPC and OXA-48 mediated resistance, which is significant for epidemiological investigations.[42]

TREATMENT ALTERNATIVES IN CARBAPENEM RESISTANT PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa is majorly involved in serious hospital-acquired infections. It builds up Sresistance to multiple antibiotics. Colistin is used as salvage therapy of *Pseudomonas aeruginosa* infections when resistance is common towards carbapenems. This drug reveals 98.8% susceptibility according to the U.S survey. According to retrospective cohort study, colistin was used in 23 seriously ill patients with multiple –drug resistance *Pseudomonas aeruginosa* infections; response was 61% with 3 patients showed resistance. Ceftolozane-tazobactam indicates excellent afficacy against many multi-drug resistant isolates. C/T was active against 95.2% CRPA (Carbapenem resistant *Pseudomonas aeruginosa*) clinical isolates. C/A (ceftazidime-avibactam) and C/T (ceftolozane-Tazobactam) are effective in case of over expression of oprD, efflux pumps and chromosomal ampC among non carbapenemase-producing CRPA clinical isolates.[43] Thirty seven patients were treated for bacteremia with carbapenem resistant (CR) pseudomonas *aeruginosa*. Among these patients, 65% of isolates exhibited multi-drug resistance.[44]

MEASURES REQUIRED TO OVERCOME CARBAPENEM RESISTANCE

Resistance towards carbapenem is identified as one of the greatest threats to human health over the World. Almost 2 million Americans per year suffer from nosocomial infections, leading to 99,000 deaths as a result of antibiotic resistance. Coordinated intrusion should be designed to get better use of antibiotics. Unecessary and inappropriate antibiotics should be avoided. We should discontinue the use of antibiotics for rapid growth and prevention of diseases in animals as well as in crops. The intimidation must be controlled by strict infection control policies and guidelines planned for every country. Skillful identification of carbapenemase productions by microbiology laboratories plays a vital role in infection control.[45] Therapy of resistant pathogen should be selected with regarding pharmacokinetics and pharmacodynamics.[46] *Pseudomonas aeruginosa* resistant genes have been detected globally, especially in Europe, South Asia and America. Hence there is an urgent need for infection control stewardship policy to control dissemination of resistance.

CONCLUSION:

Pseudomonas aeruginosa has the capacity of transferring antibiotic resistant genes to susceptible population of microorganisms.

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