Review Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SECONDARY METABOLITES FROM MANGROVE ENDOPHYTES: THEIR SIGNIFICANCE AGAINST CARBAPENEMASE RESISTANT ENTEROBACTERIACEAE – A REVIEW

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ABSTRACT

The emergence of Carbapenem-resistant Enterobacteriaceae (CRE) causes high mortality rate and is one of the most important medical issue worldwide including developing countries. The resistant pattern of CRE seems towards lactam antibiotics, including the third generation of cephalosporin except aztreonam and clavulanic acid due to the presence of resistance pattern in mobile genes. Consequently, we are in the urge to develop a new therapeutic drug against CRE to reduce the high mortality rate. Endophytes play an important role in developing a novel drug for various diseases. Recently, many researchers are working on endophytic fungi against multi-drug resistant strains to develop the novel drug. Therefore, the present review focuses on developing a novel drug using mangrove endophytic secondary metabolites against superbug - CRE.

KEYWORDS: CRE, endophytes, secondary metabolites, novel drug.

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INTRODUCTION

Carbapenem-resistant Enterobacteriaceae (CRE) or Carbapenemase-producing Enterobacteriaceae (CPE) is gram-negative bacteria that are resistant to the carbapenem class of antibiotics which is considered as the "drug of last resort" for various infections. Experts labeled the CRE as the new "superbug".[1] CRE causes life-threatening disease and associated with high mortality rates.[2] The emergence of CRE has become a severe public health issue worldwide, in the past decade.[3] The risk of CRE infections frequently seen in the patients who were admitted to hospitals, nursing homes and other healthcare settings requires facilities like ventilators (breathing machines), urinary (bladder) catheters or intravenous (vein) catheters which are taking long courses of certain antibiotics than in healthy people. As a result, most of the bacteria have become resistant to most available antibiotics. Infections with these microorganisms are very difficult to treat.[4] CRE spreads rapidly among worldwide thus we are in urge situation to treat CRE infections using fast and accurate detection in laboratories.[5] Currently, there are no consensus recommendations for screening, detection and confirmation of CRE either on the clinical or the laboratory side. The rapid detection of carbapenem resistance is crucial for guiding the treatment of the individual patient and also for instituting proper infection control measures to limit the spread of the organism.[6] Carbapenemases are enzymes which hydrolyze the β-latam antibiotics, Some bacteria belong to Enterobacteriaceae family can produce β-lactamase (carbapenemase) which hydrolyses carbapenems drugs includes ertapenem, doripenem, imipenem and meropenem etc and are resistant to penicillins, aztreonam and third-generation cephalosporins like ceftriaxone, cefotaxime and ceftazidime. These enzymes encoded by the bla genes are usually located on mobile genetic elements facilitating horizontal transmission between different gram-negative species and have been mainly reported in Enterobacteriaceae family including *E.coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii.*[6] The KPC (*Klebsiella pneumonia* carbapenemase), VIM (Verona Integron-Mediated Metallo-β-lactamase), NDM (New Delhi Metallo-beta-lactamase) and OXA-48 (oxacillinases) types are frequently found on mobile genetic elements and have the potential to spread widely.[7] Thus, the prevention of both CRE transmission and CRE infections have become challenging problems in infectious diseases. This review describes the potential of mangrove endophytes against CRE infections.

PREVALENCE OF CRE

The emergence and rapid spread of CRE worldwide which causes a major public health issue. The majority of nosocomial and community-acquired infections are caused by the family Enterobacteriaceae like *E.coli, Pseudomonas* and *Acinetobacter species*.[8] The type of CRE is based on the country which it occurs and this makes a difference in their prevalence across the European countries. Since the population migrates across high prevalence rate countries through cross-border transfer, travel and medical tourism etc. ie, OXA-48 spreads from North Africa to France and Belgium; similarly NDM-1 of Indian origin spreads from Turkey and UK to Germany.[7] The National Nosocomial Infection Surveillance System/National Healthcare Safety Network data during 2001 to 2011 stated that CRE had increased ten-fold from 1.6% to 10.4% and four-fold from 1.2% to 4.2% in *Klebsiella* species and *Enterobacter* species respectively. The CRE infection was reported in the first half of 2012 in the USA as 4% of acute-care hospitals and 18% of long-term acute-care hospitals (LTACHs).[9] The prevalence of CRE is highest in Greece, India and US in the range of 20-50%, 4-24% and 4-11% respectively.[10] The emergence of KPC in health care settings is a significant challenge to all health care professionals. KPC first emerged in North Carolina in 1999 and it was later reported in 2001. In 2013, 42 States has been documented in 42 States which lead to the endemic levels in 6 States. In 2010 overall mortality for patients infected with KPC was 23% in 7 days, 42% in 30 days, and 60 percent by the end of their hospitalization. KPC had spread rapidly in the United States as well as around the world. Endemic in areas such as the northeastern United States, Israel, Colombia, and Greece, KPC colonization is routinely found in patients in both acute- and long-term–care facilities. But it has been reported that studies of community-onset infections with KPC-producing organisms have been rare.[11] NDM-1 was first identified in 2008 in a *K. pneumoniae* isolate recovered from a Swedish patient who has been previously hospitalized in New Delhi, India. Since then, NDM carbapenemases are the focus of worldwide attention due to the rapid dissemination of the blaNDM-1 gene among Enterobacteriaceae and *Acinetobacter* species. The reservoir of those NDM producers is mainly located in Southeast Asia where the rate of carriers is estimated to be ca. 20%.[12] The prevalence of OXA-48 type enzymes increased worldwide and CTX-M ESBLs reaching pandemic proportions.[13]

CARBAPENEMASE ENZYMES AND ITS GENETIC PLATFORM

The CRE is rapidly spread in humans through the contaminated hands, foods, water, contaminated materials in hospitals etc; thus the bacteria will swap their genetic material by lateral gene transfer through transposons, integrons and plasmids.[9] Gene epidemics are caused due to the dispersal of transposons and integrons. Ultimately, that leads to new genetic and biochemical mechanisms such as plasmid addiction or hypermutability (especially with fluoroquinolones). As a result, today more than 300 types of ESBLs were reported particularly in Enterobacteriaceae family.[14] Carbapenemase enzymes strongly inhibited by clavulanic acid and aztreonam antibiotics and the combination drug works against the carbapenemase hence, it is suggestible that clavulanic acid and aztreonam antibiotics were used as the choice of drug in case of CRE.[8]

CLASSIFICATION OF CARBAPENEMASES AND THEIR GENE LOCATION

The carbapenemase enzymes are classified based on functional and molecular properties. The classification based on molecular properties includes classes of A, B, C and D. ie,

(i) serine carbapenemases-class A penicillinases and class D oxacillinases
(ii) metallo-β-lactamases-class B carbapenemases

Carbapenemase enzymes and its genetic platform were depicted in table 1.The serine carbapenemases-class A penicillinases incudes KPC, GES, SME and IMI/NMC enzymes in which KPC, GES encoded with plasmid whereas SME, IMI/NMC genes encoded with chromosomes. KPC and GES presents in transposable plasmids. Thus their prevalence rate was high and frequently reported in *Klebsiella pneumonia* and other genus of Enterobacteriaceae. Metallo-β-lactamases-class B carbapenemases includes IMP, VIM, GIM, SIM, NDM enzymes and reported in Enterobacteriaceae family and some other gram negative bacteria includes *Pseudomonas aeruginosa*. The genes under belongs to class-B were encoded with plasmid/chromosomes and they were located in transferable genes hence the prevalence rate was high especially with NDM genes in India. Class D oxacillinases are called as metallo-β-lactamases or OXA β-lactamases which hydrolyze oxacillin groups of antibiotics. The enzyme OXA encoded with plasmid/chromosomes.[8]

Table 1
Carbapenemase enzymes related with gene location.

Types of Carbapenemases	Class	Name of enzyme	Atoms in active site	Name of gene	Inhibited by			Gene location
					CLA	EDTA	ATM	
serine carbapenemases	class A	penicillinases	serine	bla-KPC	+	-	R	Plasmid
				bla-GES	+	-	S	Plasmid
				bla-SME	+	-	R	Chromosome
				bla-IMI/NMC	+	-	R	Chromosome
	class D	oxacillinases	serine	bla-OXA-23	±	-	S	Plasmid/Chromosome
				bla-OXA-24	±	-	S	Plasmid/Chromosome
				bla-OXA-58	±	-	S	Plasmid/Chromosome
				bla-OXA-48	±	-	S	Plasmid/Chromosome
metallo-β-lactamases	Class B	class B carbapenemases	Zinc	bla-IMP	-	+	S	Plasmid/Chromosome
				bla-VIM	-	+	S	Plasmid/Chromosome
				bla-GIM				
				bla-SIM				
				bla-NDM	-	+	S	Plasmid/Chromosome

Table 1 describes that the different classes of carbapenemase enzymes and their inhibitory action on ATM, CLA and EDTA[8]. ATM-aztreonam; CLA-clavulanic acid; R-resistant; S-susceptible.

CURRENT TREATMENT FOR CRE

The KPC spreads rapidly through inter-institutional transfer between the patients, leads to severe infection which requires appropriate management in the health care systems.[11] The resistance pattern of multi-drug resistant strains varies based on the locality and antibiotic regimen. The antibiotic resistant pattern of CRE was given in table 2.

Table 2
Previous and Current Clinical and Laboratory Standards Institute Interpretive Criteria for Carbapenems and Enterobacteriaceae

Agent	Previous breakpoints (M100-S19) MIC (µg/ml)			Current breakpoints (M100-S22) MIC (µg/ml)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Doripenem	-	-	-	≤1	2	≥4
Etrapenem	≤2	4	≥8	≤0.5	1	≥2
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4

Table 2 describes that the previous and current breakpoints of MIC value on carbapenem drugs. Most of the carbapenem drugs showed resistant against CRE strains.[15]

ENDOPHYTIC FUNGI FROM MANGROVE PLANTS

Marine environment is one of the most important sources for natural products in drug discovery.[16,17] Mangroves are woody plants which grow in between the range of land and sea in tropical or subtropical areas thus create exclusive ecological environments.[3]

The endophytic fungi residing inside the tissues of plants without any harm to the host plant and are mutualistic symbiont.[18] Due to their ecological diversity, most of the endophytic fungi belongs to ascomycetes and anamorphic fungi. Nearly 300,000 plant species have been discovered and each plant having one or more endophytes.[19] Nowadays endophytic fungus and their secondary metabolites are under recognizable vision towards their exclusive biological activity. The natural products from endophytic fungus play a vital role in drug discovery because they have novel and promising bioactive compounds.[18] There are 250,000 marine endophytes from 2840 marine species out of which 20,057 metabolites were isolated.[16]There are numerous marine endophytic fungi have been isolated so far which includes *Phomopsis species*[17]*, Aspergillus species*[18]*, Pestalotiopsis species*[20,21], *Pseudolagarobasidium acaciicola*, *Rhytidhysteron rufulum, Guignardia* species[22], Acremonium *strictum*[23]*, Penicillium species*[3,24,25,26]*, Lasiodiplodia* species[27] from various mangrove plants. Among which at least one or two bioactive compounds were isolated from their effective activity against various diseases. Taxol, an anticancer drug produced by endophytic fungi *Taxus brevifolia* isolated from yew tree Taxus species, for example, *Pestalotiopsis microspora* isolated from *Taxus wallichiana* produced taxol. Endophytic strains produce the same bioactive compounds as plants which will reduce the chances of harvesting plants especially slow growing tree like a yew tree.[28]

SECONDARY METABOLITES OF MANGROVE ENDOPHYTIC FUNGI

Endophytic fungi are rich sources of novel secondary metabolites.[15,17] The investigation of natural products includes isolation, identification and structural elucidation and finally finds their biosynthetic pathway at the molecular level.[19] Many bioactive compounds had different biological activities like cytotoxic[17,23,29], antioxidant[17], antifungal[17,30], antibiofilm[18], antibacterial[20], antiproliferative[24,29], antidiabetic[27], anticancer, anti-PTP1B and anti-SIRT1, anti-microtubule[31], neuroprotective activity[3] etc. The crude extract of endophytes was obtained by the fermentation process in which the fermentation broth was extracted with ethyl acetate or acetone v/v extraction for three times gives a good yield. Secondary metabolites and its biological activities were listed in the table 3.The crude extract was subjected to chromatographic analysis using silica gel and then with Sephadex column for purification and was then eluted with solvents based on the polarity.[17] The molecular formula of the compound was established using HRESIMS and the spectral analysis was done with ^{1}H, ^{13}C NMR, and 2D NMR (HMBC, HSQC, and NOESY) so that we can identify the functional group of the compounds. The structure was confirmed by X-ray crystallographic analysis.[3,24,27] The presence of oxetane ring in the natural products makes the compound identity and exclusive. Oxetane found mostly in terpenoids like oxetanocin A, dictyoxetane, merrilactone A, bradyoxetin and taxol.[22]

Table 3
Secondary metabolites and its biological activity

S.No	Compound name	Plant name	Species	Biological activity	References
1	Phomopsidone A, phomopsidone	K. candel (L.)	Phomopsis sp. A123	cytotoxic, antioxidant and antifungal	[17]
2	Flavipesins A and B, phenyl dioxolanone,	Acanthus ilicifolius	Aspergillus flavipes AIL8	antibiofilm activity	[18]
3	PestalotiopyronesA–H, pestalotiopisorin A, pestalotiollides A–B, pestalotiopinA, pestalotiopamides A–D, nigrospora pyrone D, 2-anhydromevalonic acid and p-hydroxy benzaldehyde	Rhizophora mucronata	Pestalotiopsis JCM2A4	antimicrobial and anti-proliferative against L-5178-Y, PC-12, and Hela cells	[21]
4	Guignardins A-F	Kandelia candel	Guignardia sp. KcF8	antidiabetic, antimicrobial, anticancer, anti-PTP1B and anti-SIRT1	[22]
5	Farinomaleins C–E (3–5), isoindoline congener	Avicennia marina	unidentified endophytic fungus	cytotoxicity against mouse lymphoma cell line L5178Y, antibacterial	[29]
6	Pestalotiopyrones I–L, (6S,10S,20S)-hydroxypestalotin	Sonneratia caseolaris	Pestalotiopsis virgatula	antibacterial	[20]
7	60-hydroxypestalotiopsone C, acropyrone, bicytosporone D, waol acid and pestalotiopene C	Rhizophora apiculata	Acremonium strictum	cytotoxic activity against human cisplatin sensitive, resistant A2780 cell lines, antibacterial	[23]
8	Penioxamide A, 18-hydroxydecaturin B	Rhizophora stylosa	Penicillium oxalicum EN-201	brine shrimp (A. salina) lethality	[26]
9	Deacetyl-mycoepoxydiene (DM)	not mentioned	Phomosis sp	anti-microtubule activity in MCF-7 cells	[31]
10	Dihydropyrano[2,3-c] pyrrole-4,5-dione derivative, pyranonigrin F, pyranonigrin A	Avicennia marina	Penicillium brocae MA-231	activity against aqua and plant-pathogens	[25]
11	α-Pyrone and seiricuprolide derivatives, pestalotioprolides A and B	R. mucronata, R. apiculata	Pestalotiopsis sp. PSU-MA92 and PSU-MA119	antibacterial, antifungal	[30]
12	4-(20,30-dihydroxy 30-methyl-butanoxy) -phenethanol, and 15- hydroxy-6a,12-epoxy-7b,10aH, 11bH-spiroax-4-ene-12-one	Avicennia marina	Penicillium sp. FJ-1	Antiproliferative	[24]
13	(Z)-7,40-dimethoxy-6-hydroxy-aurone-4-O-b-glucopyranoside, and(1S,3R,4S)-1-(40-hydroxyl-phenyl)-3,4-dihydro-3,4,5-trimethyl-1H-2-benzopyran-6,8-diol	Bruguiera gymnorrhiza	Penicillium citrinum	neuroprotective activity	[3]
14	β-Resorcylic acid derivatives	Acanthus ilicifolius	Lasiodiplodia sp. ZJ-HQ1	α-glucosidase inhibitory activity	[27]

Table 3 depicts the biological activity of various secondary metabolites which isolated from mangrove plants. *Penicillium* and *Pestalotiopsis species* were isolated more frequently and the rare species are also identified and isolated from *Lasiodiplodia species.* The compounds mentioned in the table can also produce by the host plant. Due to the long symbiotic relationship the endophytes can also produce the same metabolites as that of the host through recombination.[28] Few of the secondary metabolites were commercialized which listed in the table 4.

Table 4
Commercialized metabolites from marine sources

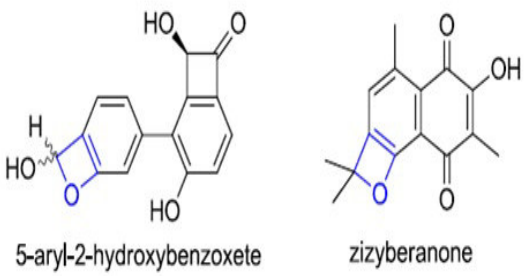
S. No.	Compound	Species	Biological Activity	References
1	α- Amylase	*Streptomyces sp., Nocardiopsis sp.*	Hydrolyse Starch	[32]
2	Carboxamycin	*Streptomyces sp.*	Antitumor; Antibacterial	[33]
3	Taxol	*Pestelotiopsis microspora*	Anticancer	[19]
4	Camptothecin	*Entrospora infrequens*	Anticancer	[34]
5	Vinblastine and Vincristine	*Alternaria species, Fusarium oxysporum*	Anticancer	[35]
6	Podophyllotoxin	*Trametes hirsuta*	Anticancer	[36]
7	AR1, AR542 and AR37 endophytes		Endophytes	[37]

The table 4 depicts that the commercialized product of marine endophytes. Endophytic secondary metabolites of mangrove plants played an important role in many fields includes medicine like anticancer, anti-inflammatory, antifungal, antibacterial, etc in agriculture like biofuel production, phytoremediation and industries like enzymes etc. AR1, AR542 and AR37 endophytes were used on perennial ryegrass and tall fescue grasses in Australia. These grasses were makes infects with fungal endophyte *Neotyphodium* for animal feed.[37] Twenty approved marine drugs and in clinical or preclinical trials were organized accordingly field collected source and biosynthesis pathways origin source.[16]

ADVANTAGES OF EXPLORING ENDOPHYTES OVER MEDICINAL PLANTS

The endophytes have many advantages over the plant metabolites. The endophytes are readily renewable for example the harvesting of 38,000 yew trees can produce only 25 kg of taxol to treat 12,000 patients.[35] The production of 1 kg paclitaxel requires 10,000-kg bark.[38] 1 g of vincristine production it requires 500 kg of leaves (1 oz of drug - 12–15 tons).[39] Also, the plant takes several years to attain a suitable growth phase for product accumulation while the endophytes are ubiquitous in nature, readily reproducible, can have long self-life of strains that the microorganisms can be preserved without losing the posterity and inexhaustible than vulnerable and critically endangered a certain number of plant species can be protected. The endophytes give high yield in the short term period.[30]Endophytes are ubiquitous in nature and present in every plant, due to the mutualistic symbiont the endophytes can produce the same phytochemical agent with that of the higher plants. Hence, we can stay away from harvesting slow growing or endangered plants for the development of the drug. For example Taxol, an anticancer drug produced by the plant *Taxus wallichiana* which can also be produced by the endophytic fungi *Pestalotiopsis microspora*.[19] The antibacterial compounds periconicins A and B were produced from the higher plants *Taxus cuspidate* and the endophytic fungi were *Aspergillus fumigatus* CY018.[40] A novel depsidone, phomopsidone A, excelsione and isobenzofuranones were isolated from the mangrove-derived endophytic fungi which possess antioxidant, antifungal and cytotoxic activities. These compounds possess oxetanes in its structure which give rigidity and leads to be like a natural product. The natural products include zizyberanone and 5-aryl-2-hydroxybenzoxete have the oxetane rings in its structure (fig 1).[17] Therefore, the mangrove endophytes can produce the same metabolites as the natural products and hence we can apply the same principle against CRE.

Figure – 1[17]
Structure of oxetane rings



Blue ring indicates the presence of oxetane.

ENDOPHYTES AGAINST MULTI-DRUG RESISTANT (MDR) STRAINS

Multi-drug resistant strains have been causing major health issues worldwide. The research on new antibacterial agent should be developed to reduce the complications of MDR strains. Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus faecalis* (VRE) are the two most important antibiotic resistant strains which were associated with hospital acquired infections. Vancomycin, teicoplanin, arbekacin, linezolid, daptomycin, tigecycline and high level gentamicin have been developed to treat MRSA and VRE infections but the strains acquire resistance pattern have been increasing towards newer antibiotics.[41,42] Natural products like Mangroves endophytes are the chemical compound produced by the living organisms; they play a major role in discovering novel secondary metabolites against various infections including multi-drug resistant strains. Investigations on mangrove (Aegiceras corniculatum) endophytic fungi *Colletotrichum* active against two MDR strains includes *Klebsiella pneumoniae* and *Acinetobacter baumanii* and their dose range was 4 µg/ml and 0.5 µg/ml respectively, whereas *Guignardia* inhibits *K. pneumonia* with the range of 8 µg/ml.[43] Some novel antibiotics were produced by endophytic bacteria includes Ecomycins (antifungal), Pseudomycins (antifungal), Munumbicins (antibacterial -effective against multiple-drug-resistant (MDR) *Mycobacterium tuberculosis,* antimalarial), Kakadumycins (anticancer). Munumbicin produced by *Streptomyces* NRRL 30562 was isolated from *Kennedia nigriscans* active against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE). Xiamycins produced by *Streptomyces sp*. strain GT2002/1503, an endophyte from the mangrove plant, *Bruguiera gymnorrhiza* are effective against MRSA and VRE.[44] With this review, an initiative popped up that the secondary metabolites from the mangrove endophytes can also work against CRE.

CONCLUSION

The present review concludes that endophytic fungi may produce novel therapeutic drug against CRE since the mangrove plants have the diverse ecosystem and the endophytic fungi can also undergo various metabolic changes. MRSA and VRE strains are frequently isolated from the hospital acquired infections and the secondary metabolites from the endophytes proved to have an effective compound against such strains. Thus the mangrove endophytes have the potential source in the management of multi-drug resistant strains. Further research should be carried out to develop novel therapeutic drugs against MDR especially against carbapenem resistant strains using mangrove endophytes. The fungal endophytes can produce promising metabolites which can complete the niches in the development of a drug against CRE strains.

ACKNOWLEDGMENT

The authors would like to thank the management of VIT University for supporting the study.

CONFLICT OF INTEREST

The authors wish to declare that they have no conflict of interest.

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