Journal of Applied Pharmaceutical Science Vol. 6 (10), pp. 132-137, October, 2016
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2016.601018
ISSN 2231-3354 

Antibiotic resistance profiling of marine halophilic bacteria and haloarchaea

Vinaya D. Shinde, Rebecca S. Thombre*

Department of Biotechnology, Modern College of Arts, Science and Commerce, Shivajinagar, Pune-411005. India.

ARTICLE INFO

Article history:
Received on: 11/04/2016
Revised on: 09/05/2016
Accepted on: 24/06/2016
Available online: 29/10/2016

Key words:
Halophiles, haloarchaea, marineisolate, antibiotic resistance, plasmid, efflux pump, coastal Maharashtra, environmental microorganisms.

ABSTRACT

Drug resistance in microorganisms is an emerging phenomenon that poses challenges to public health and treatment. The reservoir of drug resistance genes found in the resistome of environmental isolates in aquatic saline systems has been augmenting continuously. The spread of drug resistance is attributed to the dissemination of drug resistant pathogens occurring in sewage and wastewater released in the marine environments like seas. Halophiles are salt loving organisms that are found naturally in these marine environments, sea water, salterns and lakes. The present study aimed at investigation of the antibiotic resistance profile of halophiles isolated from marine ecosystems of the coastal Maharashtra, India using disk diffusion method. The plausible mechanism of drug resistance in the marine halophiles was investigated by detecting plasmids and antibiotic efflux pumps using EtBr-Cart wheel assay. The haloarchaea, *Haloarcula* sp. RR14 and *Halovivax* sp. RT5 showed multidrug resistance and presence of efflux pumps (MIC_{EtBr}= 0.5 µg/ml). The resistance of gram positive halophiles was in following order: *Salimicrobium salexigens* RR5 > *Salimicrobium flavidum* RR2 > *Alkalibacillus almallahensis* RR3. Amongst gram negative halophiles, their resistance was in following order: *Halomonas smyrnensis* RR7 > *Halomonas koreensis* RR1 > *Marinobacter oulmenensis* RR6. Plasmids were detected in *Haloarcula* sp., *Halomonas*sp. and *Marinobacter* sp. The present study related to antibiotic resistance of halophiles from marine environment is significant as some of the marine halophilic isolates like *Halomonas* sp. have been reported as potential pathogens in recent times.

INTRODUCTION

The emergence of drug resistance has increased in recent times challenging the line of treatment used for diseases. Besides, drug resistance in disease causing bacteria and organisms causing nosocomial infections, the drug resistance has now spread to bacteria present in the environment. A reservoir of drug resistance genes has been accumulating in non-pathogenic environmental microorganisms found in coastal seas, estuarine environments and deep ocean water (Vaidya 2011; Da Costa *et al*., 2013). The spread of drug resistance is attributed to the release of non-disinfected wastewaters including sewage and waste water from hospitals into the marine/aquatic environment (Da Costa *et al*., 2013). A study conducted in Mumbai, India reported the spread of antibiotic resistant *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas* and *Salmonella paratyphi* B in the coastal sea waters of Mumbai (Ali and Vaidya., 2008). Similarly, a recent study reported the spread of antibacterial resistant bacteria especially Extended Spectrum β-lactamase (ESBL) producing strains in 22 rivers of America (Vaidya 2011). The dissemination of the antibiotic resistance genes from pathogens to the environmental isolates occurs via horizontal gene transfer by conjugal transfer of plasmids. There are many factors associated with the acquisition of drug resistance by environmental isolates like use and abuse of drugs, increased multi drug resistant bacteria in aquatic habitats and transfer of antibiotic resistance plasmids by conjugation or transformation. Halophilic organisms form a major portion of the microbial community that inhabits sea water (Ventosa *et al*., 1998).

* *Corresponding Author*
Rebecca S Thombre, Dept. of Biotechnology, Modern College of Arts, Science and Commerce, Shivajinagar,Pune-411005. India
mail id:rebecca.thombre@gmail.com

Halophilic organisms are salt loving organism found in the prokaryotic as well as eukaryotic kingdom. Halophiles are classified as slight (2- 5 % NaCl), moderate (5-20 % NaCl) or extreme halophiles (20 -30 % NaCl) depending on their requirement for NaCl (Oren 2008). Halophiles comprise a heterogeneous group of heterotrophic, methanogenic and photosynthetic archaea, bacteria and eukaryotes (DasSarma *et al*., 2010). Oren *et al*. (2009) have described the emended details of *Halobacteriaceae* family and till date the family comprises of 36 genera and 129 species (Oren *et al*., 1997, 2009). Halophiles are found in aquatic systems, salterns, salt lakes, marshes and have been isolated from low salinity environments like sea water (Thombre and Oke. 2015) and hypersaline environments like saltern brines (Digaskar *et al*., 2014). The metabolites produced by halophiles like ectoine, betaine, carotenoid pigments, enzymes, anticancer and antibacterial compounds have immense applications in pharmacy and biomedicine (Thombre *et al*., 2016). The occurrence of drug resistance in bacterial inhabitants of sea water and oceans is increasing because of the horizontal gene transfer of antibiotic resistant genes from pathogens that are disseminated in the sea via release of sewage in sea (Da Costa *et al*., 2013). Sea water and aquatic systems are the natural ecological habitat of halophiles. Hence in the present investigation, halophiles previously isolated from sea water of the Arabian sea, lining the Sindhudurg district in coastal Maharashtra, India were screened for the occurrence of antibiotic resistance characteristics.

Halophiles are a group of gram positive and negative bacteria belonging to different genera like *Vibrio, Micrococcus, Salimicrobium, Halomonas, Marinococcus, Flavobacterium and Planococcus* (Ventosa *et al*., 1998). Some halophiles like *Vibrio* (Yamazi *et al*., 1959) and *Halomonas*(Stevens *et al*., 2009) are known pathogens while the pathogenicity of other halophiles and haloarchaea is largely unknown (Ventosa *et al*., 1998; Stevens *et al*., 2009). As the pathogenic nature of some halophiles is reported, the study related to antibiotic resistance of halophiles from marine aquatic environment is important.

The aim of the present report was to investigate the antibiotic resistance profile of marine halophiles, to detect the presence of plasmids and to study the possible mechanism of resistance by assessing the presence of antibiotic efflux pumps.

MATERIALS AND METHODS

Microbial strains and culture conditions

The halophilic bacteria and archaea were isolated in the laboratory using enrichment culture technique from marine samples collected from West Coast of Maharashtra, India as described previously (Thombre and Oke, 2015, Digaskar *et al*., 2014).The medium used for growth of the halophilic bacteria and archaea was Sehgal and Gibbons (SG) medium supplemented with 15 % NaCl. The components of the medium included (g/100 ml): casamino acids- 0.75, yeast extract-1, KCl- 0.2, trisodium citrate- 0.3, $MgSO_4$- 2, NaCl- 15, pH: 7.2 (Sehgal and Gibbons, 1960). The incubation conditions for halophilic bacteria was 30- 37 °C for 7- 15 days and for haloarchaea was 40 °C for 5-7 days. The halophiles used in the study are enlisted in Table 1.The cultures were preserved as glycerol stocks at -20 ºC.

Table 1: Halophilic strains isolated from marine habitat of West Coast of India used for in the study.

No.	Name	Strain designation	NCBI GenBank Accession Number
1	*Halomonas koreensis*	RR1	KP712892
2	*Salimicrobium flavidum*	RR2	KP739939
3	*Alkalibacillus almallahensis*	RR3	KP739944
4	*Salimicrobium salexigens*	RR5	KP739943
5	*Marinobacteroulmenensis*	RR6	KP739941
6	*Halomonas smyrnensis*	RR7	KP739942
7	*Haloarcula sp.*	RR14	KP712895
8	*Halovivax sp.*	RT5	KP712882

Antibiotic resistance profiling of marine halophiles

The antibiotic sensitivity test was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2012) with slight modifications in terms of medium used. The medium used for the halophilic bacteria and haloarchaea was Sehgal and Gibbons (SG) medium containing 15 % NaCl (Himedia, India)instead of the standard Mueller -Hinton Agar (MHA) (Himedia, India) as MHA did not support the growth of extreme halophiles and haloarchaea. The inoculum was prepared as per the CLSI guidelines (2012). Briefly, the turbidity of the exponential phase culture was adjusted to match the turbidity of 0.5 Mc Farland standard which corresponds to 10^8 cells/ml. The incubation temperature was 37-40 °C for 5-7 days or till growth appeared. The antibiotics used were Augmentin, Norfloxacin, Nalidixic acid, Imipenem, Tobramycin, Cefoxitin, Cefoperazone and Piperacillin/Tazobactam (HiMedia, India). Presently there are no standard guidelines and interpretive criteria mentioned by CLSI for halophilic bacteria and archaea. Hence for interpreting the results of antibacterial sensitivity testing of the gram positive halophilic bacterial isolates (*Salimicrobium flavidum*RR2, *Salimicrobium salexigens*RR5 and *Alkalibacillus almallahensis* RR3)the interpretive criteria and breakpoints suggested by CLSI (2014) for gram positive *Staphylococcus aureus* was used. Similarly, for gram negative halophiles and haloarchaea (*Halomonas koreensis* RR1, *Marinobacter oulmenensis* RR6, *Halomonas smyrnensis* RR7, *Haloarcula* sp. and *Halovivax* sp.)the interpretive criteria and breakpoints suggested by CLSI (2014) for gram negative *Escherichia coli* was used. For some antibiotics (Novobiocin, Bacitracin) the interpretive criteria were not mentioned in CLSI (2014) supplementand these were interpreted as resistant or sensitive as per guidelines for halophilic archaea described by Oren *et al*., 1997, 2009.

Extraction of plasmid DNA from halophilic bacteria and archaea

The extraction of plasmid from halophilic bacteria was performed as described by Argandon *et al*., 2003. Briefly, the cell pellet of haloarchaea was suspended in 50 µl of 1M NaCl

(Himedia, India) and lysed with 200 µl SDS/OH solution (1%/0.2M). After mixing gently for 10 min, the solution was treated with 150 µl potassium acetate and mixed by inversion and centrifuged at 10,000 rpm for 5 min using a refrigerated centrifuge (C-24 Remi, India). The supernatant was precipitated with two volumes of chilled ethanol, the plasmid is obtained after centrifugation and visualized by agarose gel electrophoresis as described by Argandon *et al*., 2003.

Antibiotic efflux pump assay

The antibiotic resistance efflux pumps produced in halophilic bacteria and haloarchaea were detected by modification of Ethidium Bromide- agar Cartwheel method (Martins *et al*., 2011). Briefly, the saline suspension of the halophiles (Absorbance adjusted to 0.5 of Mc Farland standard) were streaked on SG agar with 15 % NaCl supplemented with EtBr (Sisco Research Laboratories, India)(0 to 2.5 mg L^{-1}) divided into radial sectors and incubated at 37- 40°C for 5-7 days till growth appeared. The plates were examined under UV transilluminator and the minimum concentration of EtBr (MIC_{EtBr}) that produced fluorescence was recorded. The experiment was performed in triplicates.

RESULTS AND DISCUSSION

Halophiles are extremely resistant to different stresses like perchlorate, temperature and salinity (Chitnis and Thombre, 2014). The natural and acquired resistance to antibiotics in halophiles is also an intriguing feature of paramount importance. With the rising occurrence of drug resistance in bacteria at alarming rates, the treatment strategies and containment of the multi drug resistant strains (MDR) poses a great challenge. The drug resistance in microorganism is attributed to many factor. The ability to produce enzymes like β-lactamases, alterations of cell wall permeability and chromosomal mutations and activation of efflux pumps are few of the mechanism of drug resistance (Mapara *et al*., 2015). The most common mechanism by which bacteria acquire drug resistance is plasmids. Dealing with plasmid mediated resistance is more difficult as the antibiotic resistance genes can be easily transferred between different populations leading to release of the drug resistant genes in environmental population. Besides plasmid, the presence of antibiotic efflux pumps is also a common strategy adapted my microorganisms to garner resistance to antibiotic drugs. The present investigation focused on profiling the antibiotic resistance in halophiles and exploring for the presence of plasmids and efflux pumps in them. The halophilic isolates from marine ecosystems were profiled for their antibiotic resistance and the results are summarized in table 2.

As observed from Table 2, all the halophilic bacteria and haloarchaea were resistant to ampicillin, ciprofloxacin, bacitracin and chloramphenicol. The haloarchaea were resistant to all the antibiotics. Though there are no standard CLSI guidelines for the classification of drug resistance in haloarchaea, as the standard definition of multidrug resistance is the ability to be resistant to minimum one drug in more than three classes of antibiotics, the haloarchaea investigated in the present study can be considered to be multidrug resistant strains. However, the classification of drug resistance due to the variations in cell wall and inherent mechanisms of drug resistance in haloarchaea is still a subject of contemplation. The marine halophiles also showed marked resistance to antibiotics. *Salimicrobium* sp. and *Alkalibacillus almallahensis* were resistant to many antibiotics like ampicillin, streptomycin, bacitracin, ciprofloxacin and tetracycline (Table 2).*Halomonas* sp. was resistant to ampicillin, streptomycin, bacitracin, ciprofloxacin, tetracycline, erythromycin, chloramphenicol and gentamicin (Table 3). Similar antibiotic resistance was observed in gram negative halophile *Marinobacter oulmenensis*. Most halobacteria are sensitive to bacitracin and resistant to penicillins, cycloserine, kanamycin and neomycin (Ghosh *et al*., 2010). The haloarchaea, *Haloarcula* sp. RR14 and *Halovivax* sp. RT5 were resistant to almost all antibiotics. The antibiotic resistance was in the following order:Haloarchaea (RT5, RR15) >*Halomonas* sp. >*Marinobacter* sp. > *Salimicrobium* sp. >*Alkalibacillus* sp.

Table 2: Antibiotic resistance profile of gram positive marine halophilic bacteria by disk diffusion method**.** [Legend: R-resistant, I – Intermediate, S- Sensitive; Interpretive criteria of **Staphlococcus aureus; #Enterococci* as per guidelines of CLSI M100-S24 for disk diffusion. Isolates:*Salimicrobium flavidum*RR2, *Alkalibacillus almallahensis* RR3, *Salimicrobium salexigens*RR5].

Name of antibiotic	Concentration (µg/ml)	Class	Interpretive Criteria for Zone Diameter* (mm)			Antibiotic resistance profile of marine halophilic isolates		
			S	I	R	RR2	RR3	RR5
Ampicillin	10	β-Lactam	≥ 17	-	<16	R	R	R
Streptomycin	25	Aminoglycoside	≥ 10	7-9	≤6	R	S	R
Bacitracin	10	Polypeptide	-	-	-	R	R	R
Novobiocin	30	Aminocoumarin	-	-	-	R	S	R
Ciprofloxacin	5	Fluoroquinolone	≥ 21	16-20	≤15	R	R	R
Tetracycline	30	Tetracycline	≥ 19	15-18	≤14	R	R	R
Erythromycin#	15	Macrolides	≥ 23	14-22	≤13	S	S	R
Chloramphenicol	30	Phenicol	≥ 18	13-17	≤12	R	R	R
Trimethoprim	25	Folate pathway inhibitor	≥16	11-15	≤10	R	S	R
Gentamicin	10	Aminoglycoside	≥15	13-14	≤12	S	S	S

Table 3: Antibiotic resistance profile of gram negative marine halophilic bacteria and haloarchaea by disk diffusion method. [Legend: R-resistant, I – Intermediate, S- Sensitive; Interpretive criteria of* *Enterobacteracieae {E. coli};*#*Enterococci* as per guidelines of CLSI M100-S24 for disk diffusion. Isolates: *Halomonas koreensis* RR1, *Marinobacteroulmenensis* RR6, *Halomonas smyrnensis* RR7, *Haloarcula* sp.RR14, and *Halovivax sp*.RT5].

Name of antibiotic	Concentration (µg/ml)	Class	Interpretive Criteria for Zone Diameter* (mm)			Antibiotic resistance profile of marine halophilic isolates				
			S	I	R	RR1	RR6	RR7	RR14	RT5
Ampicillin	10	β-Lactam	≥ 17	14-16	≤13	R	R	R	R	R
Streptomycin	25	Aminoglycoside	≥ 15	12-14	≤11	R	R	R	R	R
Bacitracin	10	Polypeptide	-	-	-	R	R	R	R	R
Novobiocin	30	Aminocoumarin	-	-	-	S	S	R	R	R
Ciprofloxacin	5	Fluoroquinolone	≥ 21	16-20	≤15	R	R	R	R	R
Tetracycline	30	Tetracycline	≥ 15	12-14	≤11	R	R	R	R	R
Erythromycin	15	Macrolides	≥ 23	14-22	≤13	R	S	R	R	R
Chloramphenicol	30	Phenicol	≥ 18	13-17	≤12	R	R	R	R	R
Trimethoprim	25	Folate pathway inhibitor	≥16	11-15	≤10	S	R	S	R	R
Gentamicin	10	Aminoglycoside	≥15	13-14	≤12	R	R	R	R	R

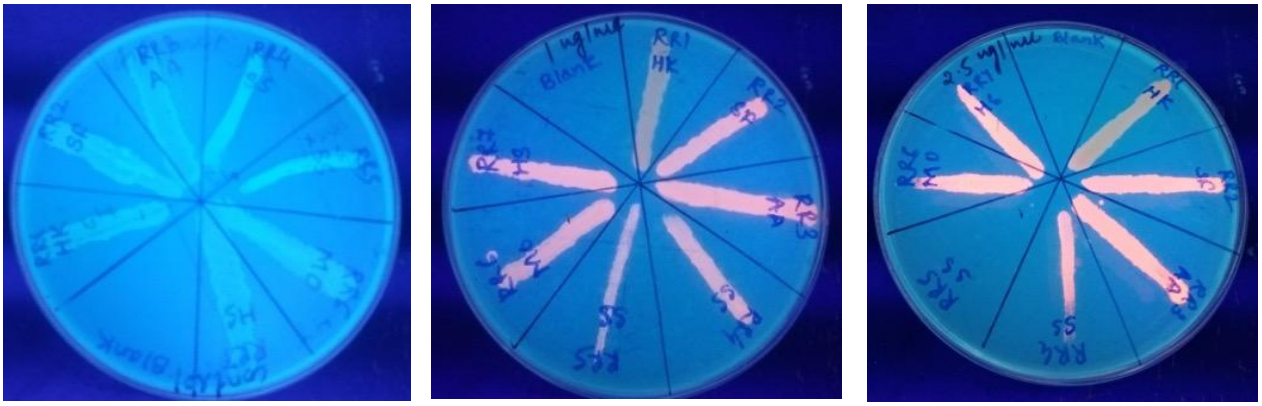

Fig 1: Detection of antibiotic efflux pump in halophiles by Ethidium Bromide Cart-Wheel Assay. (Plate 1: Control, Plate 2: SG with 1 µg/ml EtBr, Plate 3: SG medium with 2.5 µg/mlEtBr).

The acquisition of drug resistance is generally by natural genetic transformation as a mechanism of horizontal transfer of drug resistance genes (Vaidya 2011). The bacteria can acquire the genes either by transformation by uptake of naked DNA from the environment pool or by transfer of plasmids by conjugation. In either case, the transfer of genes from the pathogens released in natural econiches to environmental isolates is inevitable (Vaidya 2011). The presence of plasmids is commonly associated with antimicrobial resistance in bacteria and most of these extrachromosomal replicons harbor drug resistance genes. Plasmids and mega plasmids have been reported in halophiles earlier (Montserrat *et al*., 2003).The presence of plasmids has previously been reported in halophiles isolates from tannery waste (Ghosh *et al*., 2010).The presence of plasmids that may be the plausible underlying mechanism of drug resistance in these marine isolates was studied and the results are shown in Table 4.Plasmids were detected in *Haloarcula* sp., *Halomonas* sp. and *Marinobacter*sp. demonstrating that drug resistance may be plasmid encoded in these isolates. As *Halomonas* sp. is a known pathogen, the detection of plasmid in this drug resistant marine isolate is a significant finding. Amongst the other mechanisms of drug resistance is the overproduction of antibiotic efflux pumps. The detection of efflux pumps by Ethidium bromide Cart-wheel assay is depicted in Table 4 and Fig. 1. Efflux pumps are membrane proteins found in gram positive and negative bacteria that have the ability of extrusion of antibiotics from within the cells. Presence of efflux pump is often linked to antimicrobial resistance in bacteria (Martins *et al*., 2011).

Table 4: Presence of antibiotic efflux pumps and plasmid in halophilic isolates.

Isolate no	Name of halophilic marine bacteria and haloarchaea	Presence of antibiotic efflux pump	MIC EtBr µg/ml	Presence of plasmid DNA
RR1	*Halomonas koreensis*	-	0	+
RR2	*Salimicrobium flavidum*	+	1	-
RR3	*Alkalibacillus almallahensis*	+	1	-
RR4	*Salimicriobiumsalexigens*	+	1	-
RR5	*Salimicriobiumsalexigens*	+	1	-
RR6	*Marinobacter oulmenensis*	+	1	+
RR7	*Halomonas smyrnensis*	+	1	+
RR14	*Haloarcula* sp.	+	0.5	+
RT5	*Halovivax* sp.	+	0.5	-

There are five known major categories of efflux pumps: ABC (ATP binding cassettes) transporters, MATE (Multidrug and toxic efflux) pumps, MF (Major facilitator) pumps, SMR (Small Multidrug Resistance) pumps and RND (Root Nodulation and Division) pump(Webber and Piddock, 2002). Halophiles may

contain any of these pumps and multidrug resistant strains are known to overexpress these efflux pumps. It was observed that most halophiles had the presence of antibiotic efflux pumps which is a significant finding as over expression of efflux transporters is associated with multidrug resistance in bacteria.

The rising menace of MDR strains has instigated researchers to shift focus on the search for natural methods for treating drug resistance. For combating resistance in bacteria, alternative approaches like application of plant extracts, seed extracts and nanoparticles is being explored (Awallelu *et al*., 2013, Thombre *et al*., 2013, Francis *et al*., 2014).

Novel potential phyto-therapeutic agents against multiple drug resistant bacterial strains like plant based extracts have been studied for their potential application in reversal plasmid-mediated-resistance (Kumar *et al*., 2013). Drug resistance is now not restricted to pathogens but has now wide spread occurrence in environmental organisms.Environmental microorganisms are considered to play an active role in biodegradation, bioremediation, bio-geo chemical cycles and production of metabolites. Theincreasing occurrence of antibiotic resistance or MDR strains in environmental microorganism needs to be explored to unravel the probable reasons of natural and acquired drug resistance in them.

In the current investigation, the occurrence of antibiotic resistance in halophiles from marine environment were studied.It was found that these marine isolates were resistant to a variety of antibiotic and most of the isolates showed presence of plasmids and efflux pumps. This highlights the potential role of these organisms as reservoirs of drug resistant genes and increasing augmentation of resistome in the environmental microbiome.

CONCLUSION

It can be concluded from the present results that the halophilic bacteria and archaea from marine ecosystems were extremely resistant to a wide spectrum of antibiotics. Plasmids were detected in few marine halophilic isolates. The antibiotic resistance may be due to the presence of R- plasmids. Antibiotic efflux pumps were also detected in the marine isolates. The study indicates that the occurrence of antibiotic resistance and multi drug resistance is not limited to only pathogenic bacteria or organisms involved in hospital acquired infections but is also wide spread in marine halophilic bacteria isolated from their natural environment.

ACKNOWLEDGEMENTS

Authors thank the Principal, Modern College, Pune-5 for providing the facilities to carry out the project work. We are grateful to Dr. Kanekar P.P. for providing standard antibiotic disks.

Financial support and sponsorship: We thank Indian Space Research Organization-Space Technology Cell (ISRO-STC) for providing the financial assistance for the project.

Conflict of Interests: There are no conflicts of interest.

REFERENCES

Ali MM, Vaidya VK. Antibiotic resistance in pathogens isolated from the coastal marine environment of Mumbai. Bionano Frontier, 2008; 2:115–23.

Awallelu K, Thombre R, and Borate A. Antibacterial activity of*Crossandrainfundibuliformis* and *Jasminumsambac* against cell phone bacteria. Cent Euro J Exp Bio, 2013; 2 (1):7-11.

Chitnis A, Thombre R. Study of stress proteins induced by perchlorate and temperature stress in extremely halophilic archaea. Journal of Protein and Proteomics, 2014; 5: 49-50.

Clinical Laboratory Standards Institute. 2012. Performance standards for antimicrobial disc susceptibility tests, Twenty second Informational supplement, Tech. Rep. M100-S22, CLSI. Wayne, Pa, USA, 32: 50–60.

Clinical Laboratory Standards Institute. 2014. Performance standards for antimicrobial disc susceptibility tests, Twenty fourth Informational supplement, Tech. Rep. M100-S24, CLSI. Wayne, Pa, USA, 34: 1–230.

Da Costa PM, Loureiro L, Matos AJF. Transfer of Multidrug-Resistant Bacteria between Intermingled Ecological Niches: The Interface between Humans, Animals and the Environment. *International Journal of Environmental Research and Public Health*. 2013;10(1):278-294. doi:10.3390/ijerph10010278.

DasSarma P, Coker JA, Huse V, DasSarma S. 2010. Halophiles, industrial applications. In FlickingerMC, ed, Encyclopedia of Industrial Biotechnology. New York: John Wiley,1-9.

Digaskar V, Thombre R and Oke R. 2015. Screening of extremely halophilic archaea for its biotechnological potential. Int J Pharm BioSci,2015; 6:811-819.

Francis G, Thombre R, Parekh F and Lekshminarayan P. Bioinspired Synthesis of Gold Nanoparticles Using *FicusBenghalensis* (Indian Banyan) leaf extract. ChemSciTrans, 2014; 3(1):470-474.

Ghosh R, Pijush C, Baudhhadeb C, DebasishP. Antibiotic resistance profile of halophilic microorganisms isolated from tannery effluent. IndJBiotech, 2010;9 (1): 80-86.

Kumar V, Shriram V and Mulla J. Antibiotic resistance reversal of multiple drug resistant bacteria using *Piper longum* fruit extract. J App Pharm Sci. 2013; 3 (03): 112-116.

Mapara N, Sharma M, Shriram V, Bharadwaj R, Mohite KC, Kumar V. Antimicrobial potentials of *Helicteresisora*silver nanoparticles against extensively drug-resistant (XDR) clinical isolates of *Pseudomonas aeruginosa.* ApplMicrobiolBiotechnol, 2015; doi 10.1007/s00253-015-6938-x.

Martins M, Viveiros M, Couto I, Costa SS, Pacheco T, Fanning S. Identification of efflux pump-mediated multidrug-resistant bacteria by the Ethidium bromide-agar Cartwheel method. *In vivo,* 2011; 25: 171-178.

Montserrat A, Fernando M, Inmaculada L, Emilia Q, Ana M. Megaplasmids in Gram-negative, moderately halophilic bacteria. FEMS Microbiol Lett,2003; 81-86.

Oren A, Ventosa A, Grant WD. Proposed minimal standards for description of new taxa in the order Halobacteriales. Int J SystBacteriol,1997; 47: 233-238.

Oren A. Microbial life at high salt concentrations: phylogenetic and metabolic diversity Saline Systems, 2008; *4*:2 doi:10.1186/1746-1448-4-2.

Oren A, Arahal DR and Ventosa A. Emended descriptions of genera of the family Halobacteriaaceae. Int J SystEvolMicro, 2009; 59:637-642. doi 10.1099/ijs0.008904-0

Sehgal SN, Gibbons NE. Effect of some metal ions on the growth of *Halobacteriumcutirubrum.* Can J Microbiol,1960,6:165-169.

Stevens DA, Hamilton JR, Johnson N, Kim KK, Lee JS. *Halomonas*, a newly recognized human pathogen causing infections and contamination in a dialysis center: three new species. Medicine (Baltimore), 2009; 88 (4): 244-249.doi: 10.1097/MD.0b013e3181aede29.

Thombre R, JagtapR,Patil N. Evaluation of phytoconstituents, antibacterial, antioxidant and cytotoxic activity of *Vitexnegundo* and *Tabernaemontanadivartica* L. Int J Pharm Bio Sci, 2013; 4(1): 389 – 396.

Thombre R and Oke R. Study of stress proteins induced by temperature stress in extremely halophilic archaea, *Haloferax mediterranei* RT18. Int J CurrMicrobiol AppSci,2015;2:199-209.

Thombre R, Joshi V, Oke R,2016. Halophiles: Pharmaceutical potential and Biotechnological applications. In: Thangadurai D and Jeyabalan S, ed.Industrial Biotechnology: Sustainable Production and Bioresource Utilization.New Jersey, USA: Apple Academic Press Inc.111-139.

Vaidya VK. Horizontal Transfer of Antimicrobial Resistance by Extended-Spectrum β Lactamase-Producing *Enterobacteriaceae*. Journal of Laboratory Physicians, 2011; 3(1), 37–42. http://doi.org/10.4103/0974-2727.78563.

Webber MA, Piddock LV. The importance of efflux pumps in bacterial antibiotic resistance. J AntimicrobChemother, 2003; 51 (1): 9-11.

Ventosa A. Nieto JJ and Oren A. Biology of Moderately Halophilic Aerobic Bacteria. Microbiology and Molecular Biology Reviews, 1998; 62(2), 504–544.

Yamazi Y, Tsuneo K, Tsuyoshi S, Tiuichi I. Studies on pathogenic halophiles II. Salt requirement for growth and survival, Japan J Microb, 1959; 3(1): 576.

How to cite this article:

Shinde VD, Thombre RS. Antibiotic resistance profiling of marine halophilic bacteria and haloarchaea. J App Pharm Sci, 2016; 6 (10): 132-137.