Ecotoxicology and Environmental Safety 180 (2019) 749–755

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Attachment and antimicrobial susceptibility of bacterial associates of zooplanktonic copepod: Lesson for environmental safety

Sujoy Midya, Ram Kumar Ganguly, Sk Saruk Islam, Susanta Kumar Chakraborty*

Department of Zoology, Vidyasagar University, Midnapore, WestBengal, 721102, India

ARTICLE INFO

Keywords:
Aquatic ecosystem
Bacteria-zooplankton interactions
Environmental safety
Vitek 2 compact system
Pathogenic effects

ABSTRACT

The present study emphasizes on the antimicrobial susceptibility of different bacterial strains isolated from the external body surface of a commonly found zooplanktonic copepod (*Heliodiaptomus viduus*, Gurney, 1916) inhabiting both in fresh and brackish water bodies of Midnapore (West and East) Districts, West Bengal, India. Out of 62 bacterial isolated strains, 38 isolates were identified as Gram-positive while the remaining 24 isolates were found to be Gram-negative. Antimicrobial properties of all those bacterial strains were determined by Vitek 2 compact system using minimum inhibitory concentration (MIC) values. All isolated bacterial strains had exhibited differential susceptibilities against some selected antibiotics. Field Emission Scanning Electron Microscope (FE-SEM) analysis revealed the considerable association of bacteria on the cuticular body parts of the studied zooplankton. The outcomes of the present research are expected to enable health professionals in identifying two major problems -1) bacterial association with zooplankton which is so far mostly considered as a novel source of food for fish in aquatic ecosystems. 2) Selection of antibiotics as treatment measure because of the pathogenic effects of zooplankton associated bacteria on human being. This unattended arena of research is also supposed to evoke a new dimension not only because of bacteria-zooplankton interactions but also on undertaking of judicious strategies to find out proper ways and means to make the surface water suitable for the utilization by the common peoples (minimising bacterial contamination) in the context of human health and environmental safety.

1. Introduction

Zooplankton and bacteria representing two significant structural biotic components in the pelagic food web of an aquatic ecosystem ensuring transfer of energy and materials among different trophic levels (Da Rosa et al., 2017).

Although there exists a complete niche differentiation between zooplankton and bacteria in the water bodies, they undergo mutual interactions in order to drive the ecological functioning of the ecosystem (De Souza Cardoso et al., 2019).

The bacterial population present in an aquatic ecosystem as planktonic form (free floating) and also as biofilm which remains associated with the body parts of zooplankton (Lawrene et al., 1987). Attachment of bacteria with body parts of zooplankton have not only observed in crustacean zooplankton but also in other zooplanktonic fauna such as appendicularians, rotifers, and jellyfish (Flood, 1991; Selmi, 2001; Schuett and Doepke, 2009). Such interactive associations are supposed to render benefits to bacteria in respect of shelter and protection in the assurance of obtaining organic carbon from the chitinous appendages of copepods (Verschuere et al., 2000a,b). The occurrence of reactive dissolved organic carbon (DOC) in a higher amount to different chitinous appendages of crustacean zooplankton is supposed to be due to the active discharge of organic matter (autochthonous and allochthonous) and sloppy feeding habits of the zooplankton (Berggren, 2015). Such condition attracts the free-living aquatic bacteria to develop zooplankton-bacterial association by attaching themselves on the bodies of zooplanktonic assemblages (Peduzzi and Herndl, 1992; Hansson and Norrman, 1995; Moller, 2005).

As the occurrence and distribution of zooplankton in spatial and temporal scale experience distinct seasonal oscillations after being determined by the combined effects of different water quality parameters, zooplankton-bacteria interaction in variable ecological conditions cannot be determined through any conventional techniques (Folt and Burns, 1999). Many studies have dealt on a per volume base with the abundance of aquatic microbes because of their free-living existence (Tang et al., 2011; Schmidt et al., 2016). In spite of complete ecological niche partitioning between these two groups (zooplankton and bacteria), culture dependent studies depict almost similar bacterial taxa

* Corresponding author.
E-mail address: susantachakraborty@yahoo.com (S.K. Chakraborty).

https://doi.org/10.1016/j.ecoenv.2019.05.046
Received 5 March 2019; Received in revised form 22 April 2019; Accepted 14 May 2019
Available online 30 May 2019

both in water and in different body parts of zooplankton (Hansen and Bech, 1996; De Corte et al., 2014). This knowledge clearly intensifies an active exchange of microenvironment which in turn support a sustainable proliferation of microbes under a particular physio-chemical environment (Grossart et al., 2009).

Zooplankton being an important aquatic faunal components have been used by several authors all over the world including India as a model for experimental studies on ecotoxicology, ecosystem energetic, taxonomic studies and also as bioindicator organisms (Midya et al., 2018; Mallick et al., 2014; Mano H et al., 2016). As per National Rural Health Mission (NRHM) India, around 740 million rural peoples use freshwater resources for daily purposes and of which around 38 million people suffer from numerous waterborne diseases. Different Government hospitals of the state West Bengal, India recorded a severe mortality rate among the rural people due to an increasing rate of diarrhea and other enteric fatal diseases (Nandan, 2010). Nowadays, this type of outbreaks takes place because of the considerable increase in resistant bacterial isolates over multiple antibiotics (Kobayashi et al., 2015). Development of resistance to β-lactam antibiotics by different microbial strains have appeared to be an emerging problem to the health professionals to combat the spread and also resistance caused by bacterial infections. In such context, choice of proper antibiotics and their functions have now become a serious challenge to the medical fraternities.

In such context, a methodological attempt was made to note the antimicrobial susceptibilities of different bacterial strains in the aquatic systems and also to record their presence by identifying them through Vitek2, an automated instrumental technique developed for quick assessment of microbes using a wide array of enzymes. The present experimental research effort will help select effective antibiotics which are expected to provide proper medicinal supports in different bacterial related health issues.

2. Materials and methods

2.1. Analysis of physicochemical variables of water: design of experimental and field studies

Water samples and selected zooplankton (copepod:*Heliodiaptomus viduus,* Gurney, 1916) samples were collected from two water bodies (Study site-I and Study site-II). The former(S-I) represents the natural freshwater body located at Panskura, Midnapore (East) District (22°23′55.5″ N; 87°44′54.6″ E) and other is a brackish water body located at New Digha, Midnapore (East) District,(S-II) (21°64′03.5″ N; 87°57′19.7″E), West Bengal, India. The sampling processes were carried out during two contrasting seasons (late summer; April–May and late monsoon; September–October) characterised by different physiochemical parameters mostly determined by two prime meteorological variables like temperature and rainfall for two consecutive years (2014–2016). The zooplankton (*Heliodiaptomus viduus,* Gurney, 1916) has been selected because of its large size and easy availability among the study seasons in both the sites (Midya et al., 2018).

Zooplankton samples were scientifically collected from the subsurface water of the five sub-sampling sites in each of the studied water bodies, by using 25 μm mesh sized Nylobolt plankton net, mainly designed to collect larger zooplankton allowing smaller one to pass out. (Alam et al., 2006). Water temperature (°C), total dissolved solids (mg L^{-1}), salinity (PSU), dissolved oxygen (mg L^{-1}), and pH were immediately estimated with the Water Analyzer (TOWA, Japan, Model No- WQC-22A). Other water quality parameters such as alkalinity and chloride content of water were analysed by standard methods (APHA, 2005; Trivedy and Goel, 1984).

2.2. Isolation and screening

The identification of zooplankton (copepoda) was done to the lowest taxonomic level following standard literature (Battish, 1992; Dussart and Defaye, 2001). The quantitative study of zooplankton was done under a phase contrast microscope (Nikon E200) with the help of Sedgwick-rafter cell counter and the values were expressed in number per litter (Midya et al., 2018).

The collected copepod (*Heliodiaptomus viduus,* Gurney, 1916*)* samples were further concentrated, homogenized and incubated in Alkaline Peptone Water (APW) at 37 °C for a period of 18–24 h. The pellicle growth formed on the surface of APW was sub surfaced on to different selective agar media.

2.3. Identification of bacterial isolates and antimicrobial susceptibility

The bacterial isolates were cultured in a petriplate and the colonies were identified through biochemical characterization using Vitek compact 2 (BioMérieux), an automated system measuring the growth potential colorimetrically (De Cueto et al., 2004). Colonies from pure slant culture were allowed to suspend in 3.0 ml of sterile saline (0.50% NaCl). The pH of culture broth was adjusted to 6.5 with the help of an applicator stick and turbidity was maintained according to McFarland Turbidity Range (1.80–2.20) (Ganguly and Chakraborty, 2018). The Gram positive and Gram negative identification cards were inoculated with microbial suspension followed by card sealing, incubation at 36 °C and the readings were taken accordingly. Antimicrobial Susceptibility Testing (AST) cards such as AST N- 280 and AST P- 628 were used for determination of antimicrobial susceptibility of the isolates and the results were interpreted using a standardized Vitek2 compact software version 05.03. (Alaidarous et al., 2017).

2.4. Field emission scanning electron microscopy (FE-SEM) characterizations

Bacterial samples from the cultured disc were spread over the slide and fixed with 2.5% glutaraldehyde in cacodylate buffer (0.1M Cacodylate, 0.01 M $CaCl_2$, 0.01 M $MgCl_2$, 0.09 M Sucrose, and pH 6.9) for 45 min on ice followed by fixation with 1% osmium tetroxide for 45 min at room temperature. All samples were dehydrated with graded series of acetone (10, 30, 50, 70, 90 and 100%) on ice for 25 min for each step. Samples were then treated with liquid carbon dioxide and covered with 10 nm thick gold film and examined before a field emission scanning electron microscope (GEMINI ZEISS SIGMA 300) at an accelerating voltage of 5 V (Hammerschmidt et al., 2005).

2.5. Statistical analysis

Kruskal-Wallis test was applied to determine the significant difference in antibiotic susceptibility among different bacterial isolates (Ostertagova et al., 2014). Jonchkhree -Terspetree test was applied subsequently to ascertain the differences in the extent of variations of antimicrobial susceptibility among bacterial isolates (Ali et al., 2015).The computer program (statistical analysis) used for analyzing of all research findings was SPSS ver 21. The significance of the antibiotics for different classes of sensitive bacteria was assessed through the computation of nonparametric statistical analysis, Kruskal – Wallis test.

3. Results

3.1. Abiotic data

Ecological variables showed distinct seasonal variations in both the studied water bodies during the study period (July 2014–June 2016) (Table 1). At S-I, surface water temperature varied from 33.4 °C in summer to 26.6 °C in winter whereas dissolved oxygen (D.O.) concentrations were recorded to fluctuate in between 4.06 mg/l to 7.45 mg/l. Salinity in summer was as high as 1.86 mg/l while in monsoon it dropped to 0.42 mg/l. The mean pH values exhibited a variation

Table 1
Water quality parameters of the study sites.

Water parameters	S-I	S-II
Temperature (°C)	26.6–33.4	25.2–32.2
pH	6.4–7.6	6.32–7.4
Salinity (PSU)	0.42–1.86	7.23-21.52
Alkalinity (mg/l)	82.2–119	77.3–109
Total Dissolved Solids (mg/l)	1265–2648	8752–14026
DO (mg/l)	4.06–7.45	3.94–6.75
Chloride (mg/l)	112–622	5546–11184

*S-I and S-II are the two study sites.

from 6.4 to 7.6 at S-I. Chloride content of the water varied from 112 mg/l to 622 mg/l while the alkalinity of the water was found to oscillate from 82.2 mg/l to 119 mg/l. The values of mean Total Dissolved Solids (TDS) varied from 1265 mg/l to 2648 mg/l. At S-II, water temperatures fluctuated from 25.2 °C in winter to 32.2 °C in summer at S-II. Dissolved oxygen concentrations were found to remain in between 3.94 mg/l to 6.75 mg/l at this study site. Salinity in summer was as high as 21.52 mg/l while at monsoon, it dropped to 7.23 mg/l. The mean pH values exhibited a range of 6.32–7.4 representing mostly an alkaline condition. Chloride content of the water varied from 5546 mg/l to 11184 mg/l. The alkalinity of the water displayed a fluctuation from 77.3 mg/l to 109 mg/l. The values of mean Total Dissolved Solids (TDS) showed a range of variation from 8752 mg/l to 14026 mg/l. Thus environmental variables showed distinct seasonal variations in both the studied water bodies during the study period (July 2014–June 2016) (Table 1).

3.2. Bacterial abundance and identification

Bacterial isolates revealed the growth of high colony forming unit (CFU). The abundance of bacteria at S-I was 5.61 CFU/ml while the bacterial counts at Colony Forming Units at S-II were found to decrease in number (4.94 CFU/ml) (Fig. 1) (Table 2). The bacterial strains were initially grown on differential agar media followed by their biochemical mode of bacterial characterization have confirmed the occurrence of *Enterobacter cloacae complex (SR1) Klebsiella pneumonia (SR2), Enterobacter aerogenes (SR3), Aeromonas sobria (SR4)* and *Pseudomonas aeruginosa (SR5)* as Gram-negative (Table 3) and *Staphylococcus pseudintermedius* (RS1) and *Staphylococcus aureus* (RS2) as Gram-positive bacteria isolates (Table 4).

3.3. MIC determination and antimicrobial susceptibility

A vast array of antibiotics based on their efficacies towards cell wall breakdown, DNA synthesis inhibition, protein synthesis inhibition were used and the differential susceptibilities of bacteria against these wide spectra of antibiotics were observed subsequently. Among the total of

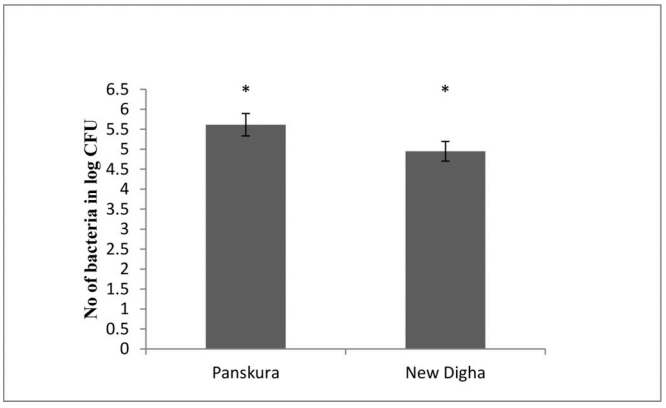

Fig. 1. The abundance of bacteria associated with copepod. Error bars represent the standard deviation where n = 3.* indicates the significance of test using Tukey test at p = 0.05.

Table 2
Abundance of Gram positive and Gram negative bacteria isolated from study sites.

Isolated bacterial species	S-I	S-II
Gram positive bacteria		
RSI	8	6
RSII	7	3
Gram negative bacteria		
SR1	8	5
SR2	4	0
SR3	4	3
SR4	2	6
SR5	5	1

Total 38 24.
* Number represents differential abundance of bacterial isolates.

Table 3
Identification of Gram negative bacterial isolates using Vitek 2 compact system.

WELL	TEST	SR1	SR2	SR3	SR4	SR5
2	ALA-PHE-PRO-ARYLAMIDASE	–	–	–	–	–
3	ADDONITOL	–	–	+	–	–
4	L-PYROLLIDIONYL ARYLAMIODASE	–	+	–	–	–
5	L-ARABITOL	–	+	–	–	–
7	D-CELLOBIOSE	+	–	+	–	–
9	BETA GALACTOSIDASE	+	+	+	–	–
10	H2S PRODUCTION	–	+	–	–	–
11	BETA-N ACETYL GLUCOSAMINIDASE	+	–	+	+	–
12	GLUTAMYL ARYLAMIDASE	–	–	+	–	–
13	D-GLUCOSE	+	–	+	+	+
14	GAMMA GLUTAMYL TRANSFERASE	+	+	+	–	+
15	FERMENTATION/GLUCOSE	+	+	+	+	–
17	BETA-GLUCOSIDASE	–	+	+	–	–
18	D-MALTOSE	+	+	+	+	+
19	D-MANNITOL	+	+	+	+	–
20	D-MANNOSE	+	+	+	+	+
21	BETA XYLOASIDASE	+	+	+	–	–
22	BETA ALANINE ARYLAMIDASE	–	+	–	–	–
23	L-PROLINE ARYLAMIDASE	–	–	–	+	+
26	LIPASE	–	+	–	+	+
27	PALATINOSE	+	–	+	–	–
29	TYROSINE ARYLAMIDASE	+	+	–	–	–
31	UREASE	–	+	–	–	–
32	D-SORBITOL	+	+	+	+	–
33	SACCHAROSE/SUCROSE	+	+	+	+	–
34	D-TAGATOSE	–	+	+	–	–
35	D-TREHALOSE	+	+	+	+	–
36	CITRATE (SODIUM)	+	+	+	–	+
37	MALONATE	–	+	+	–	–
39	5-KETO GLUCONATE	–	–	–	–	–
40	L-LACTATE ALKALINISATION	–	+	–	–	+
41	ALPHA GLUCOSIDASE	–	–	–	–	–
42	SUCCINATE ALAKALINISATION	+	+	–	–	+
43	BETA N ACETYL GLUCOSAMINIDASE	+	–	–	–	–
44	ALPHA GALACTOSIDASE	+	+	+	–	–
45	PHOSPHATASE	–	+	+	–	–
46	GLYCINE ARYLAMIDASE	+	+	–	–	–
47	ORNITHINE DECARBOXYLASE	+	+	+	–	–
48	LYSINE DECARBOXYLASE	–	+	+	–	–
52	DECARBOXYLASE BASE	–	–	–	–	–
53	L HISTIDINE ASSIMILATION	–	–	–	–	–
56	COUMARATE	–	+	+	+	+
57	BETA GLUCURONIDASE	–	–	–	–	–
58	O/129 RESISTANCE	+	+	+	+	+
59	LU-GLY-ARG-ARYLAMIDASE	–	–	–	–	–
61	L-MALATE ASSIMILATION	–	–	–	–	–
62	ELLMAN	–	–	–	–	–
64	L-LACTATE ASSIMILATION	–	–	–	–	–

+ and – denotes presence and absence of sensitivity towards these enzymes respectively.

Table 4
Identification of Gram positive bacterial isolates using Vitek 2 compact system.

Well	Test	RS1	RS2
2	D-AMYGDALIN	–	–
4	PHOSPHATIDYLINOSITOL PHOSPHOLIPASE C	–	–
5	D-XYLOSE	–	+
8	ARGININE DIHYDROLASE 1	+	+
9	BETA- GALACTOSIDASE	–	–
11	ALPHA- GALACTOSIDASE	+	+
13	ALA-PHE-PRO- ARYLAMIDASE	–	–
14	CYCLODEXTRIN	–	–
15	L-ASPARTATE ARYLAMIDASE	–	–
16	BETA- GALACTOPYRANOSIDASE	–	–
17	ALPHA-MANNOSIDASE	–	–
19	PHOSPHATASE	–	+
20	LEUCINE ARYLAMIDASE	+	–
23	ARYLAMIDASE	–	+
24	BETA- GLUCURONIDASE	–	–
25	ALPHA-GALACTOSIDASE	–	–
26	L-PYRROLIDONYL ARYLAMIDASE	–	–
27	BETA- GLUCURONIDASE	–	–
28	ALANINE ARYLAMIDASE	+	–
29	TYROSINE ARYLAMIDASE	–	+
30	D-SORBITOL	–	–
31	UREASE	–	+
32	POLYMIXIN B RESISTANCE	+	+
37	D-GALACTOSE	–	+
38	D-RIBOSE	+	+
39	L-LACTATE	+	+
42	LACTOSE	–	+
44	N-ACETYL D GLUCOSAMINE	+	+
45	D-MALTOSE	+	+
46	BACITRACIN RESISTANCE	+	+
47	NOVOBIOCIN RESISTANCE	–	–
50	GROWTH IN 6.5% NaCL	–	–
52	D-MANNITOL	–	+
53	D-MANNOSE	+	+
54	METHYL –B-D- GLUCOPYRANOSIDE	–	–
56	PULLULAN	–	–
57	D-RAFFINOSE	–	–
58	O/129 RESISTANCE	+	+
59	SALICIN	–	–
60	SACCHAROSE/SUCROSE	+	+
62	D-TREHALOSE	+	+
63	ARGININE DIHYDROLASE 2	–	–
64	OPTOCHIN RESISTANCE	+	+

+ and – denotes presence and absence of sensitivity towards these enzymes respectively.

fourteen antibiotics, amoxicillin or clavulanic acid (penicillin group) showed resistivity to all the Gram-negative isolates, whereas cephalosporins group especially cefuroxime exhibited resistivity for *Enterobacter cloacae complex,Enterobacter aerogenes, and Aeromonas sobria.*The bacterial species, *Pseudomonas aeruginosa* showed resistivity to all the cephalosporins and sulfamethoxazole with high MIC values (Table 5).These groups of bacterial isolates have been found to have a profound role in cystic fibrosis of lungs (Palmer, 2015).These results have evoked a new challenge because cephalosporin antibiotics are used as regular antibiotic formulation for treating patients of hospitals in various countries as first-line therapy for infections with a very low MIC value (< 1 μg/ml) (Adesoji et al., 2016).Pathogenic outbreaks of enterobacteriaceae were noticeably found in different respiratory, urinary and other soft tissues infections (Zhang et al., 2016; Nisijhima et al., 2007).

The bacterial strains, *Staphylococcus aureus* and *Staphylococcus pseudintermedius* exhibited resistance to penicillin group antibiotics (Benzylpenicillin and Oxacillin) at a low MIC value in contrast to eight other groups of antibiotics, whereas Sulfamethoxazole, a miscellaneous antibiotic group showed resistance at high MIC value. But five other groups of antibiotics (Aminoglycosides, Fluoroquinolone, Macrolide, Lipopeptide, and Glycopeptide) have their sensitivities against these bacterial strains (Table 6). Owing to their wide range of adaptabilities to survive in fluctuating and unstable ecological conditions vis-a-vis in hostile environments, the bacterial assemblages were found to invade any soft tissues, and skin leading to cause several syndromes (Kobyashi et al., 2015). It was found that these sorts of antibiotics have played a significant role (Asymp.Sig. = 0.978) in respect of their antimicrobial properties on different bacterial isolates. Interclass test for respective antibiotics was found to be significant by permuting them with different Gram-positive and Gram-negative bacterial isolates through Jonckheere – Terpstra test (Asymp.Sig. = 0.651). Moreover, a positive correlation (Kendall's tau-b = 0.124, Kendall's tau-c = 0.114) was found between MIC values of antibiotics and microbial isolates through Kendall rank correlation coefficient test in case of ordinal calculation as well as a Cramer's V (value = 0.314) for nominal calculation.

4. Discussion

Bacteria and zooplankton represent two separate functional groups in the aquatic ecosystem but are linked directly via nutrient flow among trophic levels through food web interactions (Tang et al., 2010). The present study has focussed on the relationship between these two distantly related biotic components inhabiting in two wetlands having contrasting ecological characteristics with the help of existing microbiological and microscopical techniques. Owing to chitin enrichment in the exoskeleton of copepods, bacteria use them as a site of attachment for proper utilization of organic carbon (C) and nitrogen (N), the major constituents of chitin and play an immense role in carbon sequestration of aquatic food chain (Nagasawa and Nemato, 1988; Carmon and Dobbs, 1997; Moller et al., 2003). Earlier studies have demonstrated that adherence and assimilation of N-acetylglucosamine within bacteria tend to focus on their adaptive strategies and behavioral manifestations for harvesting the rich source of C and N (Pruzzo et al., 2008; Meibom et al., 2004). A decrease in the diversity of bacterial strains was noticed with respect to variation in environmental conditions. This can be ascertained to salinity and chloride contents of water which were presumed to suppress the colony formation of bacteria in the brackish water body (S-II). Based on such findings, it can be inferred that the highest abundance of bacterial isolates from freshwater was due to the least salt concentration of water alongside enrichment of organic pollutants especially sewage and fecal matters from open-air defecation (Hyun et al., 1999). These observations have been corroborated with the earlier findings of Lauber et al., 2009 and Nurtjahyani et al. (2016). The present study has also unearthed information on the occurrence of an *Aeromonas sobria* as chitinolytic bacteria. Several other species of *Aeromonas* were reported previously as a source of extracellular chitinase (Saima et al., 2013). The findings of the present research in terms of zooplankton-bacteria attachment also corroborate the earlier observation of Maugeri et al., 2004. FE-SEM studies have established an exogeneous association of bacteria with the appendages, especially thoracic ones of zooplankton and also showed the impression of huge exoskeleton destruction due to high chitinase activity specifically by *Aeromonas sobria* (Fig. 2) (Pruzzo et al., 1996). Therefore, it can be suggested that except *Aeromonas sobria*, other bacterial isolates used the body of zooplankton as the shelter justifying the phenomenon on mutual relationship as commensalism. A good number of earlier research studies regarding multi-drug resistant (MDR) and extensively drug-resistant (XDR) on enterobacterial isolates have confirmed the root cause of most waterborne diseases. (Sayah et al., 2005; Kellfy et al., 2009; Graham et al., 2011).

Pathogenicity and its outbreaks disturb environmental health which is an emerging phenomenon of global concern. A significant antimicrobial resistivities were noticed with respect to different bacterial isolates which may be due to heavy metal load in a given aquatic

Table 5
Antimicrobial susceptibilities of selected Gram-negative bacteria.

Antimicrobial agents	*Enterobacter cloacae Comlpex*		*Klebsiella pneumoniae*		*Enterobacter aerogenes*		*Aeromonas sobria*		*Pseudomonas aeruginosa*	
	MIC	R/S	MIC	R/S	MIC	R/S	MIC	R/S	MIC	R/S
Penicillins										
Amoxicillin/Clavulanic acid	≤2	R	≥32	R	≤2	R	≤2	R	≤2	R
Piperacillin/Tazobactam	≤4	S	≥32	S	≤4	S	≤4	S	≤4	S
Cephalosporins										
Ceftriaxone	≤1	S	≤1	S	≤1	S	≤1	S	≥64	R
Cefuroxime	≤1	R	≤1	S	≤1	R	≤1	R	≥64	R
Cefepime	≤1	S	≤1	S	≤1	S	≤1	S	≥64	R
Carbapenems										
Ertapenem	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S
Imipenem	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Meropenem	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Fluoroquinolones										
Ciprofloxacin	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Nalidixic acid	≤2	S	≤2	S	≤2	S	≤2	S	≤2	S
Aminoglycosides										
Amikacin	≤2	S	≤2	S	≤2	S	≤2	S	≤2	S
Gentamicin	≤1	S	≤1	S	≤1	S	≤1	S	≤1	S
Tetracyclines										
Tigecycline	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S
Miscellaneous										
Sulfamethoxazole	≤20	S	≤20	S	≤20	S	≤20	S	≤80	R

S = susceptible; R = resistance; MIC = minimum inhibition concentration.

Table 6
Antimicrobial susceptibilities of Gram-positive bacteria.

Antimicrobial agents	*Staphylococcus aureus* MIC (R/S)	R/S	*Staphylococcus pseudintermedius* MIC (R/S)	R/S
Penicillins				
Oxacillin	≥0.5 (R)	R	≥4 (R)	R
Benzylpenicillin	≥4 (R)	R	0.12 (R)	R
Aminoglycosides				
Gentamicin	≤1 (S)	S	≤0.5 (S)	S
Fluoroquinolones				
Levofloxacin	≤0.5 (S)	S	≤0.12 (S)	S
Ciprofloxacin	≤0.5 (S)	S	≤0.5 (S)	S
Macrolide				
Erythromycin	≤0.25 (S)	S	≤0.25 (S)	S
Nitrofurans				
Nitrofurantoin	≤8(S)	S	≤16(S)	S
Lipopeptide				
Daptomycin	1(S)	S	1(S)	S
Glycopeptide				
Vancomycin	≤2 (S)	S	≤0.5 (S)	S
Teicoplanin	≤2 (S)	S	≤0.5 (S)	S
Miscellaneous				
Sulfamethoxazole	≥160 (R)	R	160 (R)	R
Linezolid	0.5 (S)	S	1 (S)	S

S = susceptible; R = resistance; MIC = minimum inhibition concentration.

system (Devarajan et al., 2015). Other findings suggest reduced antibiotic entry was due to enhancing DNA repair and lipid trafficking for MAR expression (Sharma, 2017). Therefore, the potential and performance of a particular antibiotic depend on the understanding of MIC value which highlights that antibiotics with less MIC value will exhibit better sensitivity of antibiotics for a given bacterial disease.

However, present research studies have recorded new roles of zooplankton in providing shelter to an array of bacteria which appeared to have pathogenic roles. Such bacterial assemblages are supposed to utilize chitin as depicted through the degradation of cuticular exoskeleton by chitinase enzymes produced by those bacteria. Thus the findings of this study are expected to open up new vista in the water quality management by way of controlling the suspected zooplankton prone to the association of bacteria and also to identify proper antibiotics to combat the problem of pathogenicity, especially in the context of developing countries where sanitation systems are not up to the mark and which thereby facilitate microbes to get access to enter the aquatic ecosystems leading to microbial pollution. Besides, out of present research studies, a number of hypotheses can be proposed as - 1) In aquatic ecosystem (both freshwater and brackish water), two major biotic components viz. zooplankton and bacteria, display their functional roles independently of each other because of complete ecological niche partitioning but are facilitated by favorable ecological conditions. They also exhibit a close mutualistic association (commensalism) wherein bacteria benefit from nutrients and shelter derived from zooplankton, especially the studied one-copepoda *Heliodoptomus viduus*. 2) In the view of the changing ecological conditions, as revealed by the seasonal variabilities of different ecological parameters (Dissolved Oxygen, pH, turbidity, salinity, chloride content) during the studied year (2014–2015), mostly determined by two important meteorological parameters (rainfall and atmospheric temperature), zooplankton-bacterial association also experiences variable association. Owing to higher salinity in the brackish water impoundment (study site-II) along with its associated ecological parameters less association and abundance of bacteria are encountered and thereby occurrence of less zooplankton bacterial association in contrast to freshwater dominated wetlands. 3) The ecological principles underlying the linkage of bacteria and zooplankton are determined not only by nutrient flow among the different aquatic trophic levels but also by the ability of chitinolytic bacteria (*Aeromonas* spp.) to produce chitinase to degrade the chitinous exoskeleton body parts of shelter providing zooplankton (copepod: *Heliodoptomus viduus*.) to harvest nutrients for bacterial growth and proliferation. 4) The bacterial strains isolated from the body parts of zooplankton revealed not only different groups (Gram positive and Gram negative) but also variable susceptibilities against selected antibiotics and antibacterial activities.

Moreover, the present research outcomes can be treated as an integrated approach towards developing knowledge on the mode of mutual interaction of two distinctly separated organisms. Bacterial characterization over these two aspects will definitely create a new vision in the context of health challenge and ecosystem safety.

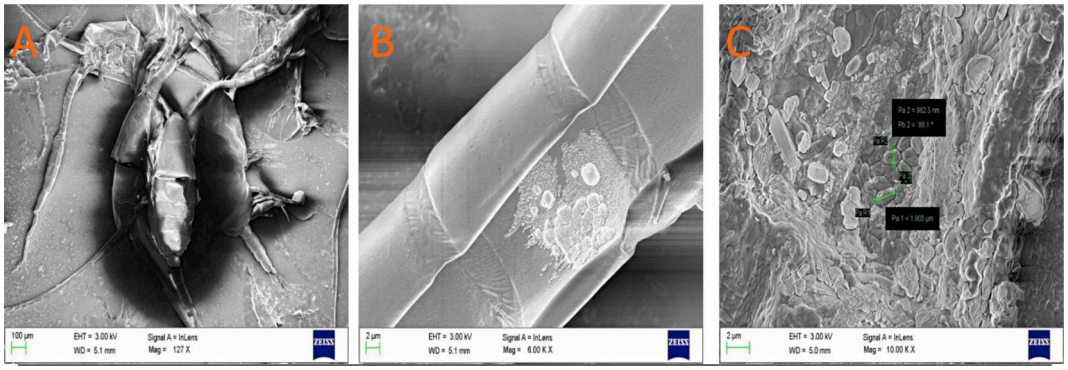

Fig. 2. FE-SEM images of bacteria attached to a copepod body surface (A: FE-SEM of the copepod, B: Attachment of bacteria to the abdomen segment, C: FE-SEM micrograph of the bacterium).

Conflicts of interest

None of the authors has any conflicts of interest.

Authors' contributions

All authors have contributed to the writing up of the present manuscript. Sujoy Midya and Sk Saruk Islam carried out sample collection, isolation, identification, biochemical characterization and data interpretation of bacterial isolates. Sujoy Midya along with Ram Kumar Ganguly was mostly involved in the identification of bacterial isolates, antimicrobial susceptibility testing, and statistical analysis.

Funding statement

No funding was granted for this study.

Acknowledgments

The authors would like to thank Department of Zoology, Vidyasagar University and Raja N.L. khan Women's College, Midnapore, West Bengal, India for supporting laboratories facilities.

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