Polymyxin Resistant, ESBL Producing *Escherichia coli* From Urban Waterbodies of Berhampur, India: A Report

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Abstract

E. coli is a Gram-negative, lactose fermenting, rod-shaped, commensal that inhabits homeothermic animals' lower gut and is also abundantly present in the environment as an opportunistic pathogen. Polymyxin is a cationic antimicrobial peptide considered the last line of antibiotics against multidrug-resistant (MDR) *E. coli*. However, polymyxin-resistant Gram-negative bacteria are increasingly becoming common in clinical isolates across the globe including India. In Odisha, there is no published report of polymyxin-resistant bacterial strains from environmental samples, even though their isolation from the clinical samples is quite abundant. In this surveillance study carried out in the urban waterbodies of Berhampur city, we found 19 polymyxin-resistant Gram-negative bacterial isolates, of which 3 were identified as *E. coli* using different biochemical and molecular biological techniques. Among the 3 *E. coli* isolates, one showed MDR phenotype and produced extended-spectrum β-lactamase (ESBL) enzyme. PCR screening for the presence of *mcr*-1, *mcr*-3, and *mcr*-9 genes yielded negative results among the *E. coli* isolates suggesting other possible mechanisms of polymyxin resistance. To our knowledge, this is the first report of MDR, ESBL-producing, polymyxin-resistant *E. coli* isolate from environmental samples of Odisha. The rise and spread of these MDR pathogens cause many life-threatening infections. These bacteria often make their way to the environment due to the lack of adequate control measures and improper management of clinical wastes. So proper management and surveillance of these bacteria is essential.

INTRODUCTION

Antibiotics are chemotherapeutic substances, produced naturally by microbes or by synthetic means and are capable of killing or hindering the growth of bacteria (Serwecińska, 2020). Antibiotics have been used successfully to combat dreadful pathogens and helped to save millions of lives. However, the indiscriminate use of antibiotics has led to the emergence of antimicrobial resistance (AMR) (Wall, 2020). AMR is the resistance of a microbe to an antimicrobial agent to which it is sensitive in its wild-type form. Resistant organisms can withstand antibiotics beyond the usual minimum inhibitory concentration (MIC) range failing the standard treatment methods. Some examples of microorganisms in this category are carbapenem-resistant *Enterobacteriaceae* including *Escherichia coli* and *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and multi drug resistant (MDR) *Pseudomonas aeruginosa* (Mancuso et al., 2021). Among these, *E. coli* is one of the most common pathogens due to its ability to quickly develop resistance to different antibiotics.

E. coli, the Gram-negative, lactose fermenting, rod-shaped, commensal in the homeothermic animals' lower gut, and is also a common inhabitant of the environment as an opportunistic pathogen. Even though most of the *E. coli* strains are avirulent, some are pathogenic and cause diseases like bacteraemia, urinary tract infections (UTIs), gastroenteritis, pneumonia, and meningitis (Mueller & Tainter, 2023) in immunocompromised and hospitalized individuals (Kaper et al., 2004). Previous reports have indicated that infections by *E. coli* are responsible for at least 1.57 lakh mortality in India in 2019 (Ikuta et al., 2022). Antibiotic resistance in *E. coli* has been reported from across the globe and increasing cases of

MDR *E. coli* are one of the greatest threats to public health, as these pathogens can transfer resistance to other bacterial species via horizontal gene transfer mechanisms (Österblad et al., 2000). Clinically several classes of antibiotics are ineffective in treating infections caused by *E. coli*, though β-lactam, quinolone, and polymyxin class of drugs are still effective. *E. coli* has the remarkable ability to acquire and produce distinct enzymes such as extended-spectrum β-lactamase (ESBL), carbapenemase, and 16S rRNA methylases etc.

Polymyxins are a group of cationic peptide antibiotics that are the last line of chemotherapeutic agents against drug-resistant Gram-negative pathogens. Polymyxins primarily target the lipid-A moiety of the lipopolysaccharide (LPS) present in the outer membrane of such bacteria (Mohapatra et al., 2021). However, due to the increasing clinical use of polymyxins resistance to them is also increasingly being reported. There is a dearth of information about the prevalence of polymyxin-resistant *E. coli* in the environment in Odisha, especially in the Southern districts. This study was conceived to address this information gap about the prevalence of polymyxin-resistant *E. coli* in the urban water bodies of Berhampur City. This study revealed the presence of polymyxin-resistant bacterial strains from all the waterbodies from where sampling was done. Moreover, the presence of a polymyxin resistant *E. coli* strain with the ability to produce extended-spectrum β-lactamase (ESBL) enzyme was detected in this study, which is to our knowledge the first report of such a strain from environmental samples in Odisha.

METHODOLOGY

Study area

The study was conducted in Berhampur, Odisha. Berhampur located at 19° 19' 0" North latitude and 84° 47' 0" East longitude (Google map), is an ancient city of the Ganjam district of Southern Odisha. This town, having a rich cultural history bears many ponds constructed during the 18th and 19th centuries, which are the lively oasis of the city. There are currently 42 ponds under the Berhampur Municipal Corporation (BeMC). These days most of the ponds of Berhampur have turned into garbage dumping and defecation sites that pose severe health hazards to the neighbourhood.

Collection of water samples

Samples were collected aseptically from 10 different waterbodies of Berhampur (Table 1). The water samples were labelled as per their locations and further processed at the Microbiology Lab, P.G. Department of Biotechnology, Berhampur University. The samples were subjected to bacteriological analysis on the same day or one day after their collection.

Table 1
The details of the sampling sites used in the study.

Sl. No.	Name of the Ponds	Location	Latitude and Longitude
1	Sunari Bandha	Santoshi Maa Mandir, Bijipur	19.3016348, 84.793548
2	Ramahari Nagar Bandha	Godavarish Nagar	19.3069356, 84.8017733
3	Dhoba Sahi Bandha	Khodasingi	19.3120366, 84.8348647
4	Beda Bandha	Gandhinagar	19.3004065, 84.7857196
5	Nilakantheshwar Bandha	Nilakantheswar Ground	19.6327196, 85.1111215
6	Bijipur Bandha	Sundar Nagar	19.3070483, 84.7932670
7	Golapalli Bandha	Manjari Nagar, New Bus Stand Road	19.3144369, 84.7987981
8	Jail /Ganesh Bandha	Indira Nagar, Industrial Estate Road	19.3004521 84.8141256
9	Gokarneshwar Bandha	Baikuntha Nagar, New Bus Stand Road	19.316141, 84.797327
10	Sai Baba Pond	Triveni Nagar	19.312275, 84.780730

Bacteriological media

For regular bacteriological work, various media such as LB broth, LB agar, EMB agar, and MacConkey agar were procured from Hi-media labs.

Bacteriological estimation by SP-SDS method

Single plate serial dilution spotting (SP-SDS) method (Thomas et al., 2015) was used to count the bacterial CFU from the water samples. In this method, the water samples were diluted serially ranging from 10^{-1} to 10^{-6}. LB agar plates divided into 8 sectors were prepared for plating of the serially diluted

samples. Then using a micropipette 20 µl of serially diluted samples were placed drop by drop with a minimal distance between each drop (15–20 drops) in the designated sector for the corresponding dilution. After absorption of the drops into the agar, the plates were incubated at 37^0C for 24 hours. Sectors that contain a countable number of colonies (6–60), were considered for CFU calculation using the following formula- CFU/mL = $n \times 5 \times 10^{d+1}$, where, n = colonies in 20 µl sample applied area, d = dilution applied in the countable sector.

To estimate the total Gram-negative bacterial count serially diluted samples were spread on MacConkey agar, and the CFU was counted as before. By subtracting the Gram-negative CFU from the total bacterial CFU, the number of Gram-positive bacteria was estimated.

Estimation of polymyxin-resistant Gram-negative bacteria

To estimate the polymyxin-resistant Gram-negative bacteria in the water samples, the samples (100 µl) were spread on MacConkey Agar plates containing polymyxin B at a concentration of 4 µg/ml. As there are no CLSI-approved breakpoints for polymyxin B for *E. coli*, a breakpoint of ≥ 4 µg/ml for resistance was used. The plates were incubated at 37°C for 24 hours, and the colonies were counted.

Selection of polymyxin-resistant bacteria with distinct morphological features

From the MacConkey plates with polymyxin B, colonies with distinct morphology (shape, size, and color) were selected for further analysis. From each pond sample, three colonies were selected in this way. These colonies were further streaked on LB agar plates supplemented with 4 µg/ml polymyxin B to obtain a pure culture. The pure cultures were maintained in agar slants and glycerol stocks for future studies.

Biochemical characterization

The isolated bacterial samples were subjected to different biochemical tests including catalase, oxidase, KOH string test, urease test, TSI agar test, and IMViC tests followed by streaking on the Eosin Methylene Blue (EMB) agar selective media to phenotypically identify the suspected *E. coli* bacterial isolates. After overnight incubation, the colonies that showed growth with green-metallic sheen were considered *E.coli* (Black, 2007). The biochemical test result of the standard *E. coli* strain was compared with the suspected polymyxin B resistant *E. coli* isolates.

Confirmatory test of suspected E. coli isolates by uid gene amplification

Genomic DNA from the suspected polymyxin B resistant *E. coli* strains was isolated following the organic extraction method. The extracted DNA samples were quantified using a Nanodrop spectrophotometer (NABI) and the quality and integrity of the DNA were analyzed by agarose gel electrophoresis. The confirmatory test for *E. coli* detection was performed by amplification of a 147 base pair region of the *uidA* (β-glucuronidase) gene as described previously (Bej, 1991). Briefly, the following PCR cyclic conditions were used to amplify the *uidA* gene; initial denaturation at 94°C for 5 mins, 35 cycles of

denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, extension at 72°C for 30 sec; and a final extension of 72°C for 5 min (Adzitey et al., 2022) in the thermal cycler (Eppendorf, Germany). The amplified DNA was run on a 2% agarose gel at 60 V. The gel was visualized using the Gel Documentation system (Bio-rad, USA).

Antibiotics susceptibility testing

Antimicrobial susceptibility testing of the suspected *E. coli* strains was performed using the Kirby-Bauer disk diffusion test. For the antibiogram, antibiotics with different modes of action belonging to different classes were obtained from HiMedia. The antibiotics tested were Carbapenem (ertapenem (10 mcg) and meropenem (10 mcg)); Glycopeptide (vancomycin (30 mcg)); Cephalosporin (cefotaxime (30 mcg) and cefepime (30 mcg)); Penicillin (ampicillin (10 mcg) and penicillin-G (10 mcg)); Quinolone (moxifloxacin (5 mcg), ciprofloxacin (5mcg), and nalidixic acid (30 mcg)); Aminoglycoside (streptomycin (10 mcg, 300 mcg) and kanamycin (30 mcg)); Tetracycline (30 mcg); Lincosamide (clindamycin (2 mcg)); Macrolide (erythromycin (15 mcg)); Rifampicin (5 mcg); Polymyxin (colistin (10 mcg) and polymyxin-B (300 units)); Sulfonamide (trimethoprim (5 mcg)); Monobactam (azoteronam (30 mcg)) and Nitrofurantoin (300 mcg). Susceptibility results were interpreted following the CLSI (Clinical and Laboratory Standard Institute) guidelines.

Determination of polymyxin minimum inhibitory concentrations (MIC)

The MIC of polymyxin B against different *E. coli* isolates was determined by broth macro-dilution method by using the cation-adjusted Mueller Hinton II broth (CAMHB) (Himedia). The bacterial strains were grown up to 0.5 McFarland standard (optical density 0.06–0.08 at 600 nm). For the broth microdilution method, the 0.5 McFarland standard inoculum was diluted 300 times and the strains were tested against a 2-fold serially diluted concentrations of polymyxin B ranging from 1 µg/ml to 512 µg/ml. The growth inhibition was checked after 20 hours of incubation at 37^0 C, shaking at 150 rpm, inside the incubator shaker (REMI).

The MIC of the antibiotics was also determined using the E-test method by placing the antibiotic strips impregnated with different concentrations of the polymyxins on the Mueller Hinton agar plate previously spread with 0.5 McFarland standard inoculum of the strains. The result was interpreted after 20 hours of incubation by checking the intersection of the zone with the strip.

Phenotypic ESBL detection by combination disk method

To detect the production of ESBL in the *E. coli* isolates a combination disk method was used as per the CLSI guidelines. Briefly, freshly prepared Mueller Hinton agar plates were spread with 0.5 McFarland standard inoculum of the *E. coli* isolates. Two antibiotic discs, cephalosporin (cefotaxime) and cephalosporin with clavulanate disc were placed on the plate and incubated overnight, and the zone of inhibition of the antibiotics discs was measured. A difference of ≥ 5 mm between the zone of inhibition

diameters of the cephalosporin disc alone and cephalosporin with clavulanate disc was considered as the ESBL production phenotype.

Plasmid isolation and mcr gene screening

The phenotypically and genotypically confirmed *E. coli* isolates were subjected to plasmid isolation by alkaline lysis method. The plasmid samples were checked by agarose gel electrophoresis (0.8%) and quantified spectrophotometrically. The purified plasmids were screened for the presence of *mcr* genes by PCR using *mcr*-1, *mcr*-3, and *mcr*-9 primers (Table 2) that have been described previously (Elizabeth et al., 2021; Li et al., 2022).

Table 2
The details of the primers used in the study for amplification of the target genes.

Primer	Sequence (5'-3')	Target Gene	Amplicon size (bp)	Reference
uidA F	AAAACGGCAAGAAAAAGCAG	β-glucuronidase	147	(Bej, 1991)
uidA R	ACGCGTGGTTAACAGTCTTGCG			
mcr-1 F	AGTCCGTTTGTTCTTGTGGC	*mcr*-1	320	(Li et al., 2022)
mcr-1 R	AGATCCTTGGTCTCGGCTTG			
mcr-3 F	AAATAAAAATTGTTCCGCTTATG	*mcr*-3	929	(Li et al., 2022)
mcr-3 R	AATGGAGATCCCCGTTTTT			
mcr-9 F	GTGTTTCCCGGCGATAAAGC	*mcr*-9	640	(Elizabeth et al., 2021)
mcr-9 R	ATCCGTTCCGTGCATGTTCT			

RESULTS

The water samples for the study were collected from 10 different water bodies (ponds) in Berhampur City, the details of which are presented in Table 1. Most of the ponds are being used for the daily activities of the people living nearby and are also used for dumping various domestic wastes.

Bacteriological estimation

When subjected to various bacteriological estimations, the pond water showed the presence of very high numbers of bacteria, and the data is presented in Table 3. The total plate count varied from 2.5×10^3 CFU/ml to 1×10^5 CFU/ml in the ponds. When checked for the Gram-negative bacterial counts estimated

on MacConkey agar plates the CFU/ml ranged from 5×10^2 to 8.5×10^4. The study also revealed the presence of polymyxin-resistant bacterial strains in almost all water bodies sampled. However, the percentage of such resistant bacteria varied from 0.04–10.3% in them (Table 3). Among the polymyxin resistant isolates both lactose fermenting and non-fermenting ones were detected on the MacConkey agar plates. The counts of such bacterial isolates are also presented in Table 3.

Table 3
The details of the bacteriological screening from the water samples. The total plate counts, Gram-negative bacterial counts, and polymyxin-resistant bacterial counts are represented as CFU/ml.

Sl. No.	Name of the Ponds	Total plate count (CFU/ml)	Gram -ve plate count (CFU/ml)	Polymyxin-resistant bacterial counts			
				CFU/ml	%	Lactose fermenting	Lactose non-fermenting
1	Sunari Bandha	6.0×10^4	4.5×10^4	1.64×10^3	2.7	7.60×10^2	8.80×10^2
2	Ramahari Nagar Bandha	6.0×10^3	5.0×10^3	2.60×10^2	4.3	9.0×10^1	1.70×10^2
3	Dhoba Sahi Bandha	9.0×10^3	2.0×10^3	9.30×10^2	10.3	8.0×10^1	8.50×10^2
4	Beda Bandha	1.0×10^5	8.5×10^4	2.05×10^3	2.0	8.80×10^2	1.17×10^3
5	Nilakantheshwar Bandha	2.5×10^3	5.0×10^2	3.0×10^1	1.2	0	3.0×10^1
6	Bijipur Bandha	2.0×10^5	4.2×10^3	8.0×10^1	0.04	3.0×10^1	5.0×10^1
7	Golapalli Bandha	6.0×10^3	5.0×10^2	3.0×10^1	0.5	2.0×10^1	1.0×10^1
8	Ganesh Bandha	6.0×10^4	5.0×10^3	5.0×10^1	0.08	2.0×10^1	3.0×10^1
9	Gokarneshwar Bandha	7.5×10^4	2.0×10^3	1.01×10^3	1.3	4.50×10^2	5.60×10^2
10	Sai Baba Pond	7.0×10^3	6.5×10^3	1.30×10^2	1.8	6.0×10^1	7.0×10^1

Characterization of polymyxin-resistant bacterial isolates

Many morphologically different colonies were observed on the MacConkey agar supplemented with polymyxin B such as red, pink, and opaque colonies based on colour; large and small colonies based on size; and mucoid colonies, fish-eyed colonies, etc (Fig. 1A). Pink-red colonies on the MacConkey medium

indicate the presence of lactose fermenting bacteria. They may include *E. coli, Klebsiella spp.*, *Citrobacter spp.*, *Enterobacter spp.*, etc. Gram-negative bacteria that grow on MacConkey agar but do not ferment lactose appear colourless or white on the medium and the agar surrounding the bacteria remains comparatively transparent (Fig. 1A). This indicated the presence of *Salmonella, Proteus, Yersinia, Pseudomonas aeruginosa* etc. A total of 19 polymyxin-resistant isolates from the MacConkey agar plates were further purified on an LB agar plate supplemented with 4 µg/ml polymyxin B and were further analyzed.

Characterization of polymyxin-resistant E. coli isolates

The 19 isolates selected for further analysis were grown on EMB agar plates, out of which 7 produced a characteristic green metallic sheen typical of *E. coli* (Fig. 1B) and a pinkish colour on MacConkey agar indicating lactose fermentation. The suspected *E. coli* isolates were subjected to amplification of *uidA* gene for confirmation. Among the 7 isolates, 3 produced the *uidA* amplicon in PCR confirming them to be *E. coli* (Fig. 2). These confirmed *E. coli* strains were named EcJB-1, EcGB-2, and EcGB-3 respectively in the subsequent analysis.

Polymyxin resistance and antibiotic susceptibility profile of the isolated E. coli strains

The MIC of polymyxin-B against the three *E. coli* isolates EcJB-1, EcGB-2, and EcGB-3 were found to be 4 µg/ml, 4 µg/ml, and 16 µg/ml respectively as determined by both E-test and broth-microdilution methods, whereas it was recorded to be 1 µg/ml for the *E. coli* control strain ATCC-25922.

To determine if the polymyxin resistance phenotype among the isolated *E. coli* strains was carried on *mcr* plasmids, they were screened for *mcr*-1, *mcr*-3 and *mcr*-9 genes by PCR. However, none of the *E. coli* strains produced a positive amplicon for the *mcr* genes indicating their absence (data not shown).

On determining the antibiotic susceptibility profile, the *E. coli* isolates were found to be susceptible to gentamicin, streptomycin, ceftazidime, nitrofurantoin, kanamycin, amikacin, and chloramphenicol (Table 4). However, resistance was observed against vancomycin, ampicillin, moxifloxacin, ciprofloxacin, nalidixic acid, clindamycin, erythromycin, rifampin, polymyxin-B, cefotaxime, and nitrofurantoin. Multidrug resistance was observed in one of the isolates (EcJB-1) as resistance was observed against three antimicrobial classes. The antibiogram of the three *E. coli* strains is presented in Table 4. Interestingly, among the *E. coli* strains one (EcJB-1) was found to be an ESBL producer that was conformed phenotypically (Fig. 1C), whereas the other two were non-ESBL producers and susceptible to 3rd generation cephalosporin class of antibiotics.

Table 4
The antibiotic susceptibility profile of the isolated *E. coli* strains as determined using the Kirby-Bauer disc diffusion test. The resistance and susceptible phenotypes are represented as R and S respectively. The *E. coli* strain ATCC 25922 has been used as a control.

Sl. No.	Antibiotic Disc	*E. coli* ATCC 25922	EcJB-1	EcGB-2	EcGB-3
1	Ertapenem (10)	S	S	S	S
2	Meropenem (10)	S	S	S	S
3	Vancomycin (30)	R	R	R	R
4	Cefotaxime (30)	S	R	S	S
5	Cefepime (30)	S	R	S	S
6	Ampicillin (10)	S	R	S	S
7	Penicillin-G (10)	R	R	R	R
8	Moxifloxacin (5)	S	R	S	S
9	Ciprofloxacin (5)	S	R	S	S
10	Nalidixic acid (30)	S	R	S	S
11	Streptomycin (10)	S	S	S	S
12	Kanamycin (30)	S	S	S	S
13	Tetracycline (30)	S	S	S	S
14	Clindamycin (2)	R	R	R	R
15	Erythromycin (15)	R	R	R	R
16	Polymyxin-B (300)	S	R	R	R
17	Colistin (10)	S	R	S	S
18	Rifampin (5)	R	R	R	R
19	Trimethoprim (5)	S	S	S	S
20	Nitrofurantoin (300)	S	R	R	S
21	Aztreonam (30)	S	S	S	S

DISCUSSION

The present study revealed that all 10 ponds that were sampled harboured polymyxin-resistant Gram-negative bacterial strains which is significant. The accumulation of these resistant bacteria may be due to the disposal of domestic and medical wastes and the presence of meat shops in the periphery could

also be a contributor. The presence of polymyxin-resistant bacteria is common in clinical samples as reported across the world including India. However, very few investigations have been undertaken in India exploring their presence in the environment. In Odisha, to our knowledge, there is no study reporting the presence of polymyxin-resistant bacterial strains in the environmental samples though they have been isolated from clinical samples.

In 2016 for the first time, colistin-resistant *P. aeruginosa* isolates were reported from Odisha (Kumar et al., 2016). Another study reported 7 colistin-resistant *A. baumannii* strains out of the 100 clinical isolates in 2017 from a tertiary care hospital in Bhubaneswar (I. C. Behera et al., 2017). Moreover, the presence of polymyxin-resistant *E. coli* (4 nos.) and *K. pneumoniae* (4 nos.) strains from 156 carbapenem-resistant *Enterobacteriaceae* (CRE) have been reported from Odisha (B. Behera et al., 2018). In the clinical samples from an ICU in Odisha presence of 15 polymyxin-resistant pathogens including *K. pneumoniae* (10), *P. aeruginosa* (3) and *A. baumannii* (1) have also been reported (Sinha et al., 2019). In 2020, colistin-resistant carbapenemase-producing *K. pneumoniae* ST70 strain in which colistin resistance determined by the *phoQ* gene mutation recovered from a patient suffering from UTI in IMS & SUM Hospital, Odisha (Sahoo et al., 2020). The presence of 27 colistin resistant *Enterobacteriaceae* out of 200 CRE clinical isolates of AIIMS, Bhubaneswar have also been reported recently (Kar et al., 2021). Clinical samples from KIMS, Bhubaneswar revealed the presence of 70 colistin-resistant isolates out of the 357 MDR Gram-negative bacterial strains (Panigrahi et al., 2022). For the first time in 2020, the presence of plasmids bearing *mcr* genes (*mcr*-1 and *mcr*-2) was reported in Odisha (Mitra et al., 2020). Interestingly, none of the reported polymyxin-resistant cases in Odisha were associated with the *mcr* gene despite its prevalence across India. The presence of polymyxin-resistant bacterial isolates from the environmental samples could be attributed to the significant number of such strains present in the clinical samples.

In this study, polymyxin-resistant *E. coli* strains were isolated from urban waterbodies of Berhampur, Odisha. *E. coli* accounted for 15.79% of the total polymyxin-resistant isolates. Out of 7 suspected *E. coli* only 3 have been confirmed by PCR. Therefore, mere confirmation of bacteria by using selective or differential media, which is the most prevalent method in the clinics may lead to incorrect interpretation.

MIC of polymyxin B against the *E. coli* strains was found to be 4-fold higher in two isolates and 16-fold higher in one isolate in comparison to the control strain *E. coli* ATCC 25922 (MIC-1 µg/ml), which is significant. The absence of the *mcr* genes in the isolates indicates the resistance property is chromosomal. LPS modification mediated by various two-component signal transduction systems (TCS), biofilm formation, porins, and efflux pumps might have contributed to the acquisition of polymyxin resistance. Surprisingly, the strains did not show colistin resistance in the antibiotic susceptibility assay though both the antibiotics belong to the same class.

Quinolone resistance was observed in the *E. coli* isolates which is a public health concern as this antibiotic has a major therapeutic importance in human medicine, livestock, pisciculture, and poultry (Velazquez-Meza et al., 2022). Quinolone resistance in *E. coli* is mainly associated with a mutation in

target enzymes DNA gyrase, Qnr plasmid-mediated resistance, decreased expression of outer membrane porins, and over-expression of multidrug efflux pumps (Klein et al., 2013).

Identification of an *E. coli* isolate which is ESBL as well as AmpC β-lactamase producer is a serious concern. This isolate also displayed resistance to cephalosporin, polymyxin, quinolone, penicillin as well as the nitrofurantoin class of drugs and was confirmed as an MDR strain. The MDR phenotype might have been accumulated by resistance plasmids or due to the presence of efflux pumps having the capacity to extrude a wide range of antimicrobial agents (Nikaido, 2009).

CONCLUSION

The rise and spread of multidrug-resistant bacterial strains causing many life-threatening diseases and associated with mortality and morbidity, is one of the major challenges in the healthcare sector. These bacteria often make their way to the environment due to a lack of control measures and improper management of clinical wastes. Polymyxins, the last resort antibiotic for many Gram-negative disease-causing, dreadful pathogens have been increasingly reported to be ineffective due to the development of resistance. In developing countries like India, the spread of polymyxin-resistant bacteria is worth considering. The presence of the mobile colistin resistance (*mcr*) gene in many cases has created an alarming situation that in a few years the drug will no longer be effective.

These days most of the ponds in the Berhampur City have turned into a cesspool, and garbage dumping sites and pose a severe health hazard to people living nearby. Furthermore, most of the ponds are present near temples and serve as a site for ritual bathing and disposal of waste. People belonging to the lower socio-economic strata also depend on these polluted water bodies and suffer from many water-borne diseases. Though the Municipal Corporation has taken many steps for the renovation of these ponds, they are not adequate to restore the water quality for safe use. Therefore, periodic microbiological surveillance of the water bodies for the presence of drug-resistant pathogens is recommended. Moreover, wastes generated from the clinics and meat shops should not be released into the water bodies.

Declarations

Competing interests: The authors declare no competing interests.

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AUTHOR CONTRIBUTIONS

SKD and SSM conceived the project; SKD and IP did the work; SKD, IP, and SSM analysed the data; SKD and SSM wrote the manuscript.

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Figures

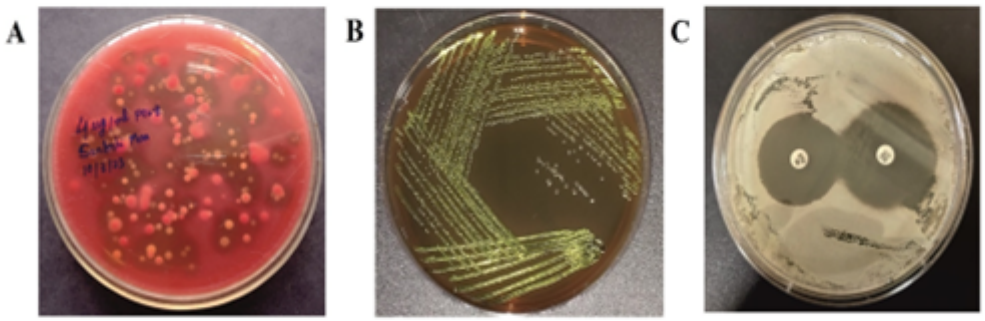

Figure 1

Representative images of the isolation of Gram-negative bacterial colonies on MacConkey agar plates supplemented with polymyxin B antibiotic (A); presumptive identification of *E. coli*strains on EMB agar plate (B); confirmation of ESBL production by the *E. coli* strain EcJB-1 using the combination disk method (C).

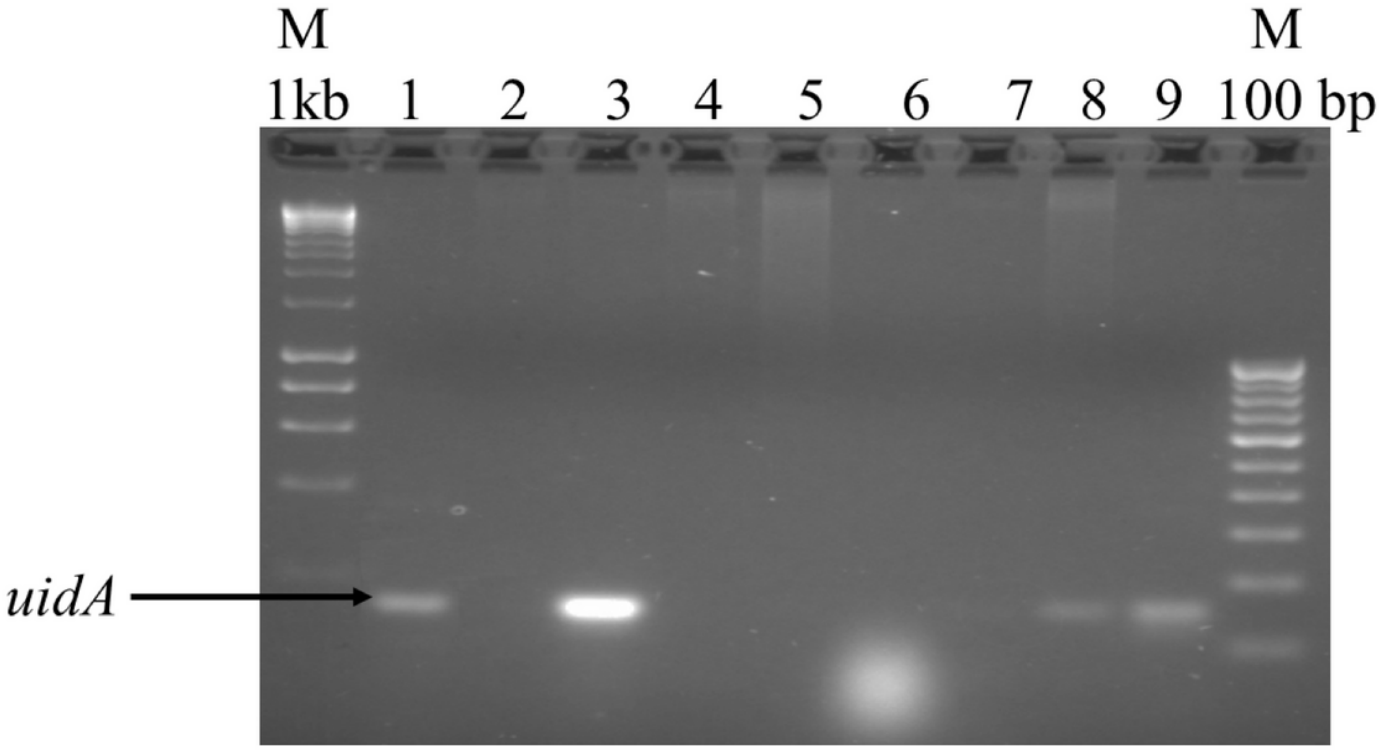

Figure 2

Agarose gel electrophoresis of the *uidA* amplicon from the presumptive *E. coli* strains for their confirmation. The sequence of the strains used in the PCR are numbered as 1-9. Strains 1, 3, and 8 are named EcJB-1, EcGB-2, and EcGB-3 respectively, whereas strain 9 is the *E. coli* strain ATCC 25922 used as a positive control in the PCR.