Original article

Predation potential of Bdellovibrio spp. against human urinary tract infections (UTIs) pathogens

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ABSTRACT

Bdellovibrio spp. are living predatory Gram-negative bacteria that predominantly prey on other Gram-negative bacteria associated with urinary tract infections (UTIs). After being isolated from UTIs patients, two UTIs bacteria were identified in this study as Escherichia coli-1 and Escherichia coli-2. Bacterial growth inhibition zones were identified, with tetracycline inhibiting E. coli-1 most effectively (26±1.53 mm) and streptomycin inhibiting E. coli-2 most effectively (27±1.53 mm). Using the "Double layer agar" technique and type strain Pseudomonas fluorescence-103 as the host, Bdellovibrio spp., which are widespread in nature, were isolated from Vaigai river water and veterinary outflow samples. The isolates were molecular level (16s rRNA) identified as Bdellovibrio sp. strains MPR 17 (MH201007.1) and Bdellovibrio sp. strains 18 (MH230068.1). The predatory capability of the isolated Bdellovibrio sp. strain MPR 17 was proved by its remarkable activity (23±2.58 mm) and (25±4.19 mm) against E. coli-1 and E. coli-2 in 100µl. Besides, Bdellovibrio sp. strain MPR 18 shown exceptional effectiveness against E. coli -1 and -2 (26±3.56 mm and 28±2.50 mm). Bdellovibrio sp. MPR 17 shown outstanding lytic activity against E. coli-1 (70 %) and E. coli-2 (68%) and Bdellovibrio sp. MPR 18 strain exhibited outstanding lytic activity against E. coli-1 (78 %) and E. coli-2 (62 %) in 2 mL. The comparison to chemical antibiotics, the predatory potential of Bdellovibrio sp. MPR 18 strain (26±3.56) is similar to the antibiotic sensitivity of E. coli-1 (26±1.53mm). However, the Bdellovibrio sp. MPR 18 strain has a higher predatory potential (28±2.50 mm) against E. coli-2 (27±1.53 mm). The results of our research indicate that the Bdellovibrio sp. MPR 17 and MPR 18 strains are successful in reducing the number of Gram-negative bacteria that infect the urinary system.

Keywords: *Bdellovibrio sp., Urinary Tract Infections, Predatory potential, Escherichia coli.*

Introduction

The emergence of bacteria that have become resistant to several drugs and for which there is currently no therapy poses a threat to the effectiveness of antibiotics, which have revolutionized modern medicine (WHO, 2021)[22]. Antimicrobial resistance (AMR), which is frequently caused by overuse of antibiotics, is a major danger to global health and the economic threat (Tillotson and Zinner, 2017)[21]. Rapidly rising the levels of antimicrobial resistance in Gram-negative bacterial pathogens and highlighted the urgent need for the development of alternative forms of antibacterial therapies (Allen, 2017)[1], World Health Organization (2021)[22] has noted several as critically urgent for new therapeutics.

Antibiotic resistance has grown to be a serious problem in recent years. The most frequent human pathogenic bacteria linked to urinary tract infections is *Escherichia coli* (Koroleva *et al*., 2023)[12]. According to Bernaitis *et al*., (2024)[2] urinary tract infections (UTIs) are becoming a more common health issue globally. To control the microorganisms that cause UTIs, we require alternate therapies.

In Russia and Eastern Europe, bacteriophages are frequently employed as antibacterial treatments (Kakasis and Panitsa, 2018)[11]. Numerous bacteriophages and predatory bacteria, such as *Bdellovibrio bacteriovorus*, demolish a lot of Gram-negative bacteria (Negus *et al*., 2017)[13]. Using live predatory bacteria, such *Bdellovibrio bacteriovorus*, which are tiny Gram-negative bacteria that are common in soil and aquatic habitats and that naturally infiltrate and destroy other Gram-negative bacteria, is one innovative way to treat illnesses. These predatory bacteria have two life stages, including an intraperiplasmic growth phase and a free-living assault phase. The *Bdellovibrio bacteriovorus* are consequently special bacteria that feed on a broad range of vulnerable Gram-negative bacteria. (Cavallo *et al*., 2021and Das *et al*., 2024)[4,5]. It has been found that *B.*

bacteriovorus HD 100 (Im *et al*., 2018)[7], *B. bacteriovorus* 109J (Sun *et al*., 2017)[20], and *M. aeruginosavorus* strain ARL-13 (Kadouri *et al*., 2013)[10] are effective against pathogens that are known to produce biofilms, including *A. baumannii*, *E. coli*, *Enterobacter spp., K. pneumoniae, Proteus spp., P. aeruginosa*, and *Staphylococcus aureus*.

B. bacteriovorus unique predation mechanism provides for its predatory selectivity against Gram-negative bacteria. When bacteriophages and *Bdellovibrio bacteriovorus* bacteria preyed on an *E. coli* colony together, the quantity of prey decreased noticeably more (Hobley *et al*., 2020)[8]. The purpose of this study was to determine whether isolated bacteria that cause urinary tract infections (UTIs) could interact with the predatory bacteria *Bdellovibrio* spp.

Materials and methods

Collection and isolation of UTIs bacteria

Urine samples were taken from patients at the Government Rajaji Hospital in Madurai, Tamil Nadu, India, who had urinary tract infections (UTIs). The membrane filtering was applied right away to the collected samples. The filtrates are being transferred to a particular MacConkey Agar medium in order to isolate Gram-negative bacteria that cause UTIs. Presumptive UTIs bacteria are selected and streaked onto nutrient agar after a day of incubation. They are then purified to produce a pure culture by incubating them for 24 hours at 37°C in an aerobic condition.

Identification of UTIs bacteria

Nutrient agar plates were used to observe the colonies colour, shape, surface, margin, and size. The hanging drop method is used to determine the organism's motility. The conventional approach was used to detect Gram staining. Several biochemical tests are used to identify the bacteria causing UTIs, including the citrate utilization test, indole test, urease test, nitrate test, and triple sugar iron agar test.

Antibiotic sensitivity assay for isolated UTIs bacteria

To control the isolated UTI pathogenic strains and determine the most effective inhibitory antibiotic, standard antibiotic discs, including Azithromycin (AZM), Erythromycin (ERY), Streptomycin (S), Ampicillin (AMP), Tetracycline (TE), Ofloxacin (OF), Amikacin (AK), and Kanamycin (K), are purchased from Hi-Media Laboratories Pvt. Ltd. in India.

Predatory assay in double layer agar

Veterinary waste water and Vaigai river water samples were collected in Madurai region, Tamil Nadu, India. The bacteria were isolated using double layer agar procedures, and they were then identified using molecular level (16s rRNA) and transmission electron microscopy examination. Double layer agar plating techniques were used to measure the cell density of each isolate (5×10^4 PFU/mL & 4×10^4 PFU/mL) using the host bacteria *Pseudomonas fluorescens*-103. Both isolated strains were diluted serially tenfold in sterile distilled water. Furthermore, 10 ml of 0.8% top agar was combined with 100 µL aliquots of serial tenfold dilutions and 100 µL of suspension of both isolated UTIs strains (1.3×10^8CFU/ml & 1.4×10^6CFU/ml). The mixture was then put onto a 1.5% (bottom agar) agar plate that had been prepared beforehand and allowed to solidify. The plaques were then recorded after the double layer agar plates were cultured for seven days at 37°C (Odooli *et al.,* 2020)[14].

Predatory assay in broth culture

In a 100 ml side-arm flask containing 20 ml of diluted nutritional broth, the bacteriolytic assay was performed in triplicate. Both obtained UTIs bacterial strains were centrifuged at 3,500 rpm for 15 minutes and re-suspended in PBS to acquire a specified OD (OD_{580}=0.50) in order to assess lysis kinetics and effective antibiotic potential. The isolated UTIs bacterial culture was combined with 2mL of both isolated predatory strains suspension at logarithmic phase. The mixture was then incubated at 30°C with shaking at 120 rpm, and the OD values were finally recorded every hour until the stationary phase. Additionally, control flasks were kept, and statistical analysis was performed on the data (Selvaraj *et al*., 2019)[19].

Results

Isolation and identification of UTIs bacteria

Two UTIs bacteria in pure culture were isolated from a particular agar medium, MacConkey Agar. Colony Forming Units (CFU) were computed to determine the zone of inhibition for UTI-1 and UTI-2 based on the growth curve (48h) recorded at 580 nm. The characteristics of the two UTIs strains rod-shaped, pink, motile, and Gram-negative were used to document them. Both isolated bacteria also showed a variety of biochemical reactions, including the triple sugar iron agar test (+, +), citrate utilization test (-, -), indole test (+, +), urease test (-, -), and nitrate test (+, +). The test pathogens were verified as Gram-negative bacteria based on the results of

the biochemical test. These distinct bacterial characteristics are therefore regarded as *Escherichia coli*-1 and *Escherichia coli*-2 microorganisms.

Antibiotic sensitivity assay for isolated UTIs bacteria

The bacterial growth inhibition zone size is evaluated and interpreted using standard procedure for the selected antibiotics. *E. coli*-1 is most effectively inhibited by tetracycline (26±1.53mm). *E. coli*-2 (27±1.53 mm) is most effectively inhibited by streptomycin. According to the findings, *E. coli*-1 TE>ERY>S>AK>AZM>OF>K>AMP and *E. coli*-2 S>TE>AK>ERY> OF>AZM>K>AMP had the highest antibiotic sensitivity.

Predatory assay in Double layer agar

Transmission electron microscopy reveals the structural information about the inner structure of both the *Bdellovibrio* sp. strains and UTIs isolates (Fig.1a &b). The isolates were molecular level (16s rRNA) identified as *Bdellovibrio* sp. strains MPR 17 (MH201007.1) and *Bdellovibrio* sp. strains 18 (MH230068.1), and their sequences were submitted to the Gene Bank. After seven days at 37°C, the predatory potential of *Bdellovibrio* sp. strains MPR 17 and 18 was shown against two UTIs bacteria. The 100µl of isolated *Bdellovibrio* sp. strain MPR 17 demonstrated exceptional activity (23±2.58 mm) and (25±4.19 mm) against *E. coli*-1 and *E. coli*-2, demonstrating the predatory potential identified. Additionally, *Bdellovibrio* sp. strain MPR 18 shown outstanding efficacy against *E. coli* -1 and *E. coli* -2 (26±3.56 mm) and (28±2.50 mm) (Fig. 2a &b).

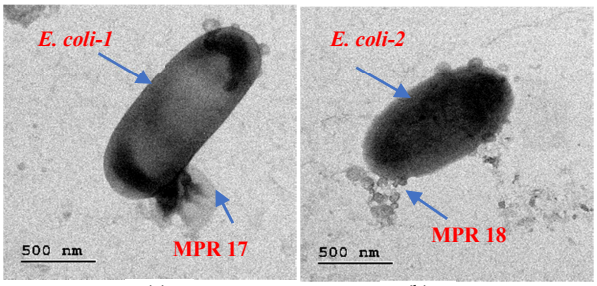
Predatory assay in broth culture

The predatory activity of the *Bdellovibrio* sp. MPR 17 strain was tested on *E. coli*-1 and *E. coli*-2 prey in nutrient broth. After 16 hours, the *Bdellovibrio* sp. MPR 17 strain reported predation curve values against *E. coli*-1 and *E. coli*-2 stabilized at OD580nm = 0.63 ±0.17 and 0.64 ±0.02. After 48 hours, turbidity level was attained against *E. coli*-1 (0.35±0.23 (70%)) and *E. coli*-2 (0.34±0.13 (68%)). Accordingly, 2 mL of *Bdellovibrio* sp. MPR 17 shown outstanding lytic activity against *E. coli*-1 (70 %) and *E. coli*-2 (68%) (Fig. 3).

After 14 and 16 hours, the *Bdellovibrio* sp. MPR 18 strain performance against *E. coli*-1 and *E. coli*-2 stabilized at OD580nm = 0.65 ± 0.04 and 0.60 ± 0.01. After 48 hours, this turbidity level was reached the OD value 0.39 ± 0.15 (78 %) and 0.31 ± 0.09 (62 %) against *E. coli*-1 and *E. coli*-2. Consequently, the *Bdellovibrio* sp. MPR 18 strain exhibited outstanding lytic activity against *E. coli*-1 (78 %) and *E. coli*-2 (62 %) in 2 mL (Fig. 4).

Predatory efficacy comparison

Using double layer agar plate techniques, *Bdellovibrio* sp. MPR 17 & 18 were used to lyse the *E. coli*-1 (23±2.58mm) and (26±3.56 mm) bacteria. Furthermore, isolated *Bdellovibrio* sp. MPR 17 & 18 effectively lysed the *E. coli*-2 UTIs bacteria, yielding values of (25±4.19 mm) and (28±2.50 mm). Tetracycline, the chemical antibiotic of choice, shows the best antibacterial activity against *E. coli*-1 (26±1.53mm). *E. coli*-2 (27±1.53 mm) is then most successfully controlled by streptomycin antibiotics. The findings show that, in comparison to chemical antibiotics, the predatory potential of *Bdellovibrio* sp. MPR 18 strain (26±3.56) is similar to the antibiotic sensitivity of *E. coli*-1 (26±1.53mm). However, compared to chemical antibiotics, the *Bdellovibrio* sp. MPR 18 strain has a higher predatory potential (28±2.50 mm) against *E. coli*-2 (27±1.53 mm) (Fig. 5).



(a) (b)

Fig. 1. (a) & (b): Transmission Electron Microscopic Structure of *Bdellovibrio* sp. MPR 17 & 18 lawn of *E.coli*-1 & 2

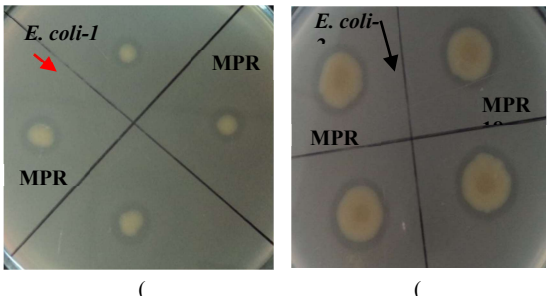


Fig.2. (a) & (b): *Bdellovibrio* sp. strain MPR 17 & 18 lawn of *E.coli*-1 & 2 with double layer agar plating techniques

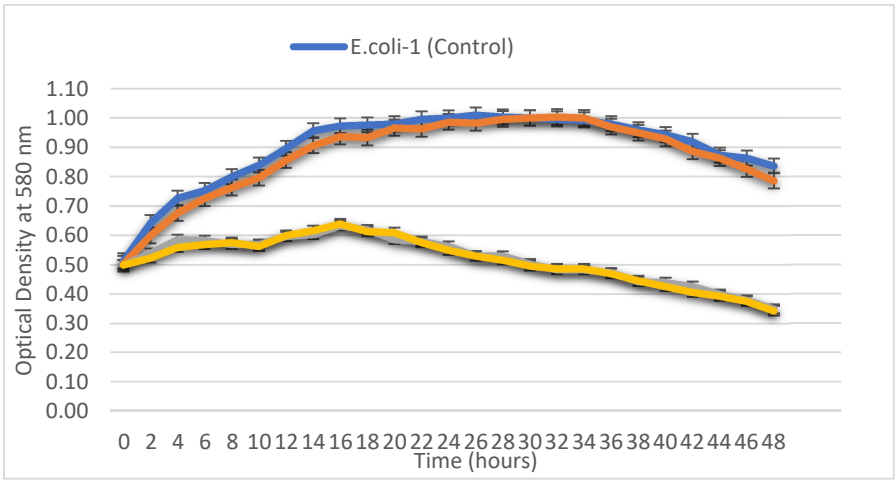


Fig.3. The Lytic Potential of *Bdellovibrio* sp. strain MPR 17 compared to effective antibiotic in broth culture of *E.coli*-1 & 2

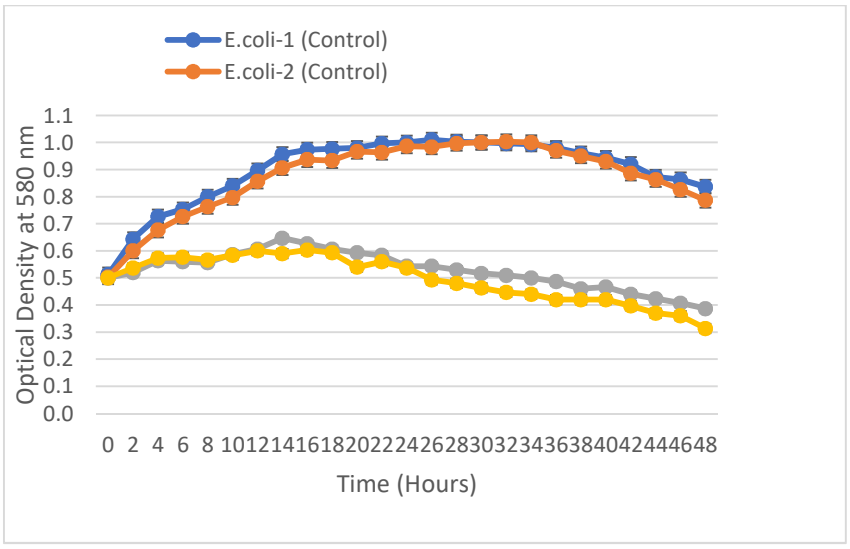


Fig.4. The Lytic Potential of *Bdellovibrio* sp. strain MPR 18 compared to effective antibiotic in broth culture of *E.coli*-1 & 2

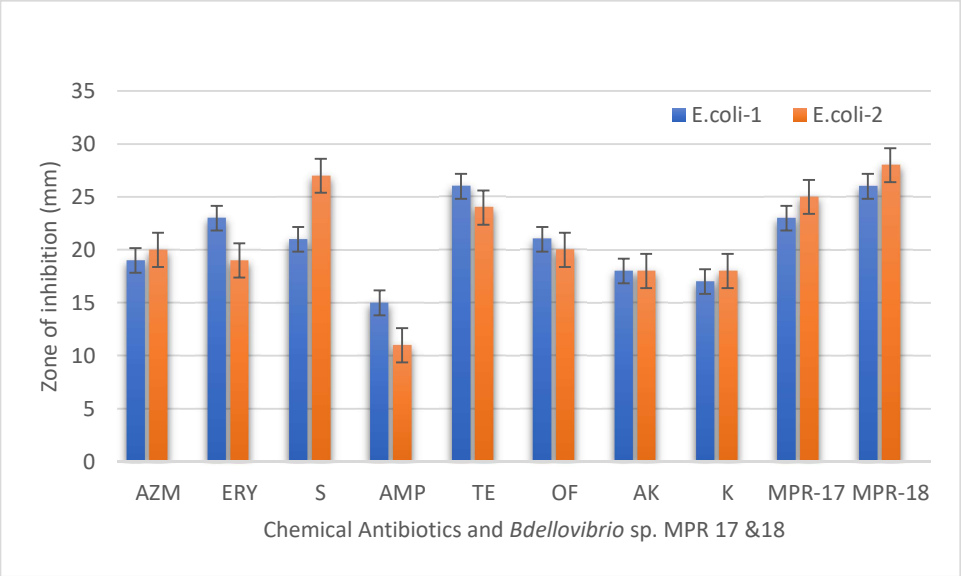


Fig.5. Predatory efficacy comparison of *Bdellovibrio* sp. strain MPR 17 & 18 with *Escherichia coli*-1 & 2
Note: Azithromycin (AZM), Erythromycin (ERY), Streptomycin (S), Ampicillin (AMP), Tetracycline (TE), Ofloxacin (OF), Amikacin (AK) and Kanamycin (K)

Discussion

Escherichia coli was the predominant uropathogen isolated from urine samples, consistent with previous reports identifying *E. coli* as the leading causative agent of urinary tract infections (UTIs). In addition to *E. coli, Staphylococcus aureus* and *Staphylococcus saprophyticus* were among the most frequently isolated bacterial pathogens, highlighting their important role in the etiology of UTIs (Prasada Rao *et al*., 2022)[17]. Furthermore, the five biochemical techniques used to identify the isolated Gram-negative bacteria include the synthesis of indole, tryptophan deaminase activity, glucose and cellobiose fermentation, and H2S. The most prevalent bacteria were *E. coli* (55.5%), *Klebsiella* spp. (23.0%), *Proteus* spp. (7.14%), *Pseudomonas* spp. (6.34%), and *Acinetobacter* spp. (3.96%), which were identified from 126 individuals with urinary tract infections (Page *et al*., 2013)[16]. In this investigation, both UTIs strains were isolated and their characteristics were noted, including rod-shaped, pink, motile, Gram-negative, and biochemical reactions using the triple sugar iron agar test (+, +), citrate utilization test (-, -), indole test (+, +), urease test (-, -), nitrate test (+, +), and urease test (-, -). The test pathogens were identified as *E. coli*-1 and *E. coli*-2 based on the results of the biochemical test. The majority of the 151 bacterial species that were isolated from patients with urinary tract infections were *Escherichia coli* (43%), *Enterococcus faecalis* (10.6%), and *Klebsiella pneumoniae* (7.3%). The majority of the 688 research participants were asymptomatic, but 144 (20.9%) had UTIs that were verified by culture (Gebremedhin *et al*., 2024)[6].

Numerous medicines, including gentamicin (67.7%), amikacin (70.8%), nalidixic acid (64.6%), ciprofloxacin (61.5%), and azithromycin (69.2%), were shown to be ineffective against *E. coli* isolates. The highest multiple antimicrobial resistance index (MARI) was found in *E. faecalis, S. xylosus*, and *E. coli,* however all bacterial isolates exhibited resistance to at least one antibacterial agent (Gebremedhin *et al*., 2024)[6]. According to our research, tetracycline inhibits *E. coli*-1 (26±1.53mm) the best. *E. coli*-2 (27±1.53 mm) is most effectively inhibited by streptomycin.

Transmission electron microscopy reveals the structural information about the inner structure of both the *Bdellovibrio* sp. strains and UTIs isolates. Using the double-layer agar method, *Bdellovibrio* sp. YBD-1 was isolated from healthy yak fecal samples. It was identified by transmission electron microscopy (TEM) and specific 16S rDNA sequencing study involving the host bacterium *E. coli* (Yao Xi *et al*., 2024)[23]. 100µl of isolated *Bdellovibrio* sp. strain MPR 17 shown outstanding activity (23±2.58mm) and (25±4.19mm) against *E. coli*-1 and *E. coli*-2 in this predatory research. Additionally, *Bdellovibrio* sp. strain MPR 18 shown outstanding efficacy against *E. coli*-1 and *E. coli*-2 (26±3.56mm) and (28±2.50mm). Additionally, filter papers containing strain YBD-1 were used to measure the inhibitory zone in various diameters at 15°C to 50°C. At 25°C to 40°C,

strain YBD-1 showed the biggest diameter (27.34±0.31) for the zone of inhibition with *E. coli* as observed within the fifth day (Yao Xi *et al.,* 2024) [23].

The *B. exovorus* MPR11-detected lytic efficiency effectively inhibits *P. mirabilis, P. syringae, M. bovis, B. glumae*, and *V. parahaemolyticus*. When compared to their effective antibiotics, Amikacin and Gentamycin, the in vitro bacteriolytic activity study results of *B. exovorus* MPR11 against *P. mirabilis* and *P. syringae* reveal 16 and 7% greater activity, respectively (Selvaraj *et al*., 2019)[19]. However, after 16 hours, the *Bdellovibrio* sp. MPR 17 strain reported predation curve values against *E. coli*-1 and *E. coli*-2 stabilized at OD580nm = 0.63 ±0.17 and 0.64 ±0.02. After 48 hours, turbidity level was attained against *E. coli*-1 (0.35±0.23 (70%)) and *E. coli*-2 (0.34±0.13 (68%)). Therefore, isolated UTIs bacterial activity decreased, resulting in the perception of excellent lytic activity. Ottaviani *et al*. (2020)[15] observed that the predatory bacterium *B. bacteriovorus* 109 J reduced the growth of *E. coli* on the chicken slices and in the canned beef by 4.3 log and 2.1 log, respectively, after 6 hours.

Additionally, *B. bacteriovorus* 109 J shown efficacy against toxigenic and multidrug-resistant strains of *E. coli* in addition to all tested strains (Ottaviani *et al*., 2020) [15]. After 14 and 16 hours, the *Bdellovibrio* sp. MPR 18 strain performance against *E. coli*-1 and *E. coli*-2 stabilized at OD580nm = 0.65 ± 0.04 and 0.60 ± 0.01. After 48 hours, this turbidity level was reached the OD value 0.39 ± 0.15 (78 %) and 0.31 ± 0.09 (62 %) against *E. coli*-1 and *E. coli*-2. Therefore, excellent lytic activity was then observed as the activity of *E. coli*-1 and *E. coli*-2 bacteria decreased. By measuring absorbance at OD600nm and using double layer plate plaque techniques, *Bdellovibrio* sp. demonstrated a specific lysis activity on a number of aquatic pathogens, including *Aeromonas veronii, Aeromonas hydrophilia, Vibrio fluvialis, Escherichia coli, Vibrio anguillarum, Vibrio cholerae,* and *Citrobacter freundii* (Yang *et al*., 2023)[9].

The results indicate that the predatory potential of the *Bdellovibrio* sp. MPR 18 strain (26±3.56mm) is comparable to the antibiotic sensitivity of *E. coli*-1 (26±1.53mm) when compared to chemical antibiotics. Nonetheless, the *Bdellovibrio* sp. MPR 18 strain shows a greater predatory potential (28±2.50mm) against *E. coli*-2 (27±1.53 mm) in comparison to chemical antibiotics. As a result, *B. bacteriovorus* created the hydrolytic enzymes, which are a new source of lytic proteins for use in the medicinal, agricultural sectors, food and biotechnology. (Bratanis *et al*., 2020)[3]. Predation strategies for *Bdellovibrio* spp. with a focus on possible novel uses of these organisms for food preservation, water treatment, industrial process enhancement, biotechnology, and antibiotics against bacteria resistant to multiple drugs. According to Selvaraj *et al*. (2018)[18], *Bdellovibrio* and like organisms (BALOs) have the potential to be used as "live antibiotics" in the fields of human, animal, plant, food, and the environment since they lyse other Gram-negative bacterial strains.

Conclusion

Urinary tract infections, which impact millions of people annually, are the most serious illness in both sexes and all age groups. Alternative medicines must be used even when the careless use of universal antibiotics to treat bacterial infections that persist causes antibiotic resistance. Using the special kind of predatory bacteria (*Bdellovibrio* sp. MPR 17 and MPR 18) that lyse the other Gram-negative bacteria is the only practical way to combat the disease-causing bacteria. The results of our study show that the effectiveness of the *Bdellovibrio* sp. MPR 17 and MPR 18 strains decreases the Gram-negative bacteria that infect the urinary system.

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