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Research Article

Potentially Pathogenic Bacteria in Water Bodies and Drinking Water Supplies in and Around Bareilly, India

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Abstract

The study was conducted to evaluate the microbial quality of water supplies in Bareilly city and nearby villages. A total of 111 samples comprising community pond water (45), drinking water (36), water tap handle swabs (city, 23) and sewage water (7, city) were analysed. Total of 363 bacterial isolates belonging to 25 genera were identified of which 71.3%, 47.7% and 30% isolates had multiple drug resistance, carbapenem resistance and produced extended spectrum-β-lactamases (ESBL), respectively. Twenty of the 36 drinking water samples had coliforms and 33.3% were positive for *Escherichia coli*. Besides, 55 samples had ESKAPE bacteria, 43.24% were positive for carbapenem resistant bacteria (CRB) and 24.3% samples had carbapenem resistant Enterobacteriaceae (CRE). In drinking water samples 8.3 % had CRE and 33.3% had CRB. Two third (65.2%) of water faucet (tap) handles in public places had CRBs mostly belonging to ESKAPE group of pathogens, and 52.2% carried CRE. The community pond water was still the bigger health hazard since 64.4% and 44.4% of samples were positive for CRB and CRE, respectively. The study indicated that community water sources either for drinking or for other purposes in and around Bareilly city were potential source of MDR, CR and ESBL producing strains.

Keywords: AMR; CRE; Carbapenem-resistance; Community Water; ESBL; MDR

Introduction

Microbial water quality specifically used for drinking and other community uses is of high significance due to potential role of water in spreading waterborne and foodborne infections. Water contaminated with potentially pathogenic and opportunistic bacteria leads to millions of death every year in under developed and developing countries. World over 4.0% of all deaths and 5.7% of all disability or ill health have their roots in poor quality water consumption; estimates reveal that contaminated drinking-water cause more than 500000 diarrhoeal deaths each year [1]. In India, during 2010-2014 contaminated water killed 13,000**,** Uttar Pradesh had maximum 3,382 deaths followed by West Bengal, Andhra Pradesh and Odisha [2]. From 2013-2017 water-borne diseases including cholera, diarrhoea, typhoid and viral hepatitis caused 10,738 deaths and more than 69.14 million cases of illness in India and loss of 73 million working days due to water-borne diseases. Among all, the bacterial infections were at the top among waterborne infections leading to more than 68.51 million cases and 8595 deaths in 2013-17 in India [3].

To monitor drinking and recreation water quality national and international standards are in force since long. The Bureau of Indian Standards (BIS) drinking water specification (BIS 10500:1991) was drawn up in 1983 and its revision dates in July 2010 (Amendment No. 3) maintained the ISI-IS: 2296-1982 maximum tolerance limit of 50 *E. coli* in 100 ml of drinking water.

In 2012, it was again revised to meet the international standards of no detectible coliforms and thermotolerant *E. coli* in 100 mL of water. However, there is no mention about other microbes and microbial quality of other than drinking water. Indian standards are now in accordance of WHO recommendations [4] i.e., there must be no *E. coli* or thermotolerant coliform bacteria in 100 mL of drinking water. Coliform bacteria are not just *E. coli* but include all lactose fermenting bacteria belonging to the genera *Escherichia, Citrobacter, Klebsiella, Enterobacter, Serratia* and *Hafnia*. Though coliform or thermotolerant *E. coli* count is generally accepted method for assessing microbial quality of water free from faecal contamination, enteric viruses and protozoa, which are more resistant to disinfection. Thus, the absence of *E. coli* may not necessarily indicate freedom from these organisms and it may be desirable to include more resistant microorganisms, such as intestinal enterococci (*Enterococcus faecalis, E. faecium, E. durans and E. hirae*), *Clostridium perfringens* spores or coliphages for testing for water quality [4]. As per International Organization for Standardization (ISO) standards, for detection and enumeration of faecal indicator bacteria in water for detection of faecal streptococci and clostridia besides membrane filtration enrichment method is recommended. Similarly for coliforms membrane filtration method is recommended over MPN tube method. Though other bacteria in drinking water may be hazardous [5] and may cause serious health problems, are not considered serious threats as most of them are susceptible for general water treatment processes including pseudomonads, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Vibrio* spp. Therefore, Heterotrophic Plate Counts (HPC) is recommended for testing of drinking water for all potential and opportunistic pathogens including *Acinetobacter, Aeromonas, Flavobacterium, Klebsiella, Moraxella, Serratia, Pseudomonas* and *Xanthomonas* [5]. Looking at the importance of microbial water quality the present study was conducted to assess the presence of potentially pathogenic and opportunistic bacteria in drinking water and associated environment and to determine their antimicrobial resistance pattern.

Materials and Methods

Sample collection

The study was conducted during March to April 2019 and a total of 111 samples comprising of pond water (city ponds 31, village ponds14), drinking water (city 17, village 19), water tap handle swabs (23, city) and sewage water (7, city) shown in the map (Figure 1) were collected for detection of bacteria as per standard method [6]. Briefly, for collection of water samples from municipal drinking water faucets in Bareilly at public places at different points in city like court, railway station, bus station, market place and from nearby villages about 2-3 L of water was allowed to flow before collecting the water sample. The samples were collected in 100 ml sterile bottles fitted with screw caps, labelled and kept in an ice box for transporting to laboratory within two hours of collection. Samples of sewage and pond water were collected in 100 mL water sterile bottles after hang dipping wide mouth bottles into the water with the help of sterile string of desired length at least from three places from a pond. Subsequently, three samples from the same pond were mixed together in to one. After collection of water, bottles were fitted with screw caps, labelled and kept in an ice box for transporting to laboratory within 2 h of collection [6]. For collection of swab samples of drinking water faucet handles, lightly moistened sterile cotton swabs were rubbed all over the handle three times on top and down faces of the handles and swabs were transferred in to sterile 50 ml test tube and screw capped to bring to the laboratory on ice within two h of collection.

Figure 1: Water sampling area map of Bareilly.

Isolation and identification of bacteria

For isolation of bacteria from water samples, one ml of water was transferred to 10 ml of buffered peptone water, incubated for 8 h at 37°C and the growth was streaked onto MacConkey agar (BBL, Diffco, USA) and Nutrient agar (BBL, Diffco, USA) plates, incubated at 37°C for 24 h and observed for isolated colonies. All

different types of colonies (2-3) were picked up and re-streaked onto nutrient agar plates for purification and incubated at 37°C for 24 h. The pure cultures were tested for morphological, culture, staining and biochemical characteristics using standard protocols [7,8]. Thereafter, bacterial isolates were classified up to genus and species using criteria laid in Bergey's Manual of Determinative Bacteriology [9].

Antimicrobial susceptibility assay

Antimicrobial sensitivity of isolates was determined using disc diffusion method following CLSI [10] guidelines using standard antimicrobial discs (Difco BBL, USA) for amoxycillin, amoxycillin + clavulanic acid, ampicillin, azithromycin, cefepime, cefotaxime, cefotaxime + clavulanic acid, cefoxitin, chloramphenicol, ciprofloxacin, colistin, cotrimoxazole, ertapenem, erythromycin, fosfomycin, gentamicin, imipenem, linezolid, meropenem, moxalactam, nalidixic acid, nitrofurantoin, oxacillin, piperacillin + tazobactam, tetracycline, tigecycline and vancomycin on MHA plates or bovine serum added MHA (for fastidious organisms like *Streptococcus/ Enterococcus* species isolates). A reference sensitive *E. coli* strain (E-382) available in the laboratory was used as control antibiotic sensitive strain. Bacteria resistant to ≥1 antibiotic of three or more classes of therapeutically used antimicrobial groups were classified as multi-drug-resistant (MDR) and those resistant to any of the three carbapenem drugs (ertapenem, imipenem, meropenem) were classified as carbapenem resistant (CR) bacteria.

For determining extended spectrum β-lactamase (ESBL) production and metallo- β-lactamase production E-test strips (Biomerieux, France) were used as per direction of the supplier. For all isolates multiple antibiotic resistance (MAR) indices were calculated by formula; number of drugs resisted/ number of drugs tested [10].

Susceptibility of bacterial isolates to herbal antimicrobials was determined for ajowan (*Trachyspermum ammi*) seed oil, betel (*Piper betle*) leaf oil, carvacrol (Sigma, USA), cinnamledehyde (Sigma, USA), Cinnamon *(Cinnamomum verum)* oil, citral (Sigma, USA), guggul (*Commiphora mukul*) oil, holy basil (*Ocimum sanctum*) oil, lemongrass (*Cymbopogon citrates*) oil, marjoram (*Origanum majorana*) essential oil, rosewood (*Dalbergia latifolia*) oil, sandalwood (*Santalum album*) oil, and thyme (*Thymus vulgaris*) oil. All herbal oils except guggul oil claiming >99.9% purity were received from Shubh Flavours and Fragrance Ltd, New Delhi while pure guggul oil was a kind gift from Dr. MZ Siddiqui, Processing & Product Development Division, ICAR - Indian Institute of Natural Resins & Gums, Namkum, Ranchi, India. The 6 mm discs loaded with 1µL of herbal compound/ oil were used for determining sensitivity of isolates through disc diffusion assay as described earlier [11]. Similar to MAR index the herabl MAR (HMAR) indices were also determined for all the isolates.

Statistical analysis

Bacterial isolates and their antimicrobial sensitivity data analysis was done using Microsoft Office Excel worksheet using statistical tools like correlation for inhibition zone diameters of different antimicrobials, odds ratio, Fisher's exact test and Chi-square test.

Results

A total of 111 samples comprising pond water (city ponds 31, village ponds14), drinking water (city 17, village 19), water faucet (tap) handle swabs (city, 23) and sewage water (city, 7) were tested for detection of bacteria. Of these, 363 isolates (84 Gram-positive, GPBs; 279 Gram-negative, GNBs) of bacteria belonging to 25 genera and 74 species (Tab. 1) were identified. *Pseudomonas* spp. dominated in occurrence and was detected in 39 samples followed by *Escherichia* spp. (32), *Aeromonas* spp. (29), *Enterococcus* spp. (21), *Klebsiella* spp. and *Staphylococcus* spp. in 20 samples each (Table 1). Many of the bacteria were detected only in a few samples (Table 1) thus their frequency of occurrence in different type of samples could not be compared. However, a few of the bacteria had more common occurrence in samples of one or more specific source type (Table 2) as enterococci detected on water tap handles were rarely detected in drinking water (p, 0.01). City pond waters were significantly richer source of aeromonads (p, <0.001), *Alcaligenes* spp. (p, 0.05), enterococci (p, 0.01) and staphylococci (p, 0.003) than ~~in~~ drinking water samples from different city points. Microbiota of city and village drinking water was almost similar but pseudomonads were more often (p, 0.015) detected in drinking water in city than in villages. Almost similar frequency of occurrence of different types of bacteria in city and village ponds was evident but *Proteus* spp. strains (p, 0.008) were isolated from village ponds only. Similar genus and species of bacteria could be detected in pond and drinking water in villages but occurrence of enterococci and staphylococci was significantly more common in pond water (p, ≤0.005) than in drinking water. Though city drinking water was common source for pseudomonas their occurrence in swage water was still more common (p, 0.01) than in drinking water in Bareilly city.

Genus of bacteria detected	Species of bacteria isolated	No. of Samples Positive	Isolates	Percent of bacteria having			
				MHADR	MDR	CR	ESBL
Pseudomonas	*P. aeruginosa 37, P. alcaligenes 1, P. fluorescens 5, P. maltofila 3, P. pseudoalcaligenes 5, P. stutzeri 1*	39	52	84.62	80.77	51.92	17.31
Escherichia	*E. blattae 1, E. coli 56, E. fergusonii 1*	32	58	43.10	44.83	25.86	39.66
Aeromonas	*A. bestiarum* 6, *A. caviae* 1, *A. hydrophila* 10, *A. jandaei* 7, *A. media* 5, *A. popoffii* 2, *A. salmonicida* 5, A. *schuberti* 7, *A. sobria* 8	29	49	59.18	67.35	42.86	48.98
Enterococcus	*E. faecalis 16, E. faecium 19, E. mindtii 2, E. pseudoavium 1, E. solitarius 1*	21	39	69.23	79.49	69.23	7.69
Klebsiella	*K. oxytoca 1, K. ozaenae 2, K. pneuminaee ssp. pneumoniae 17*	20	24	70.83	83.33	75.00	41.67
Staphylococcus	*S. arlettae 20, S. aureus 2, S. capitis 2, S. acrnosus 1, S. gallinarum 1, S. haemolyticus 9, S. intermedius 1, S. kloosii 1, S. lentus 1, S. sciuri 1*	20	39	87.18	94.87	71.79	25.64
Enterobacter	*E. aerogenes 2, E. agglomerans 16*	15	18	55.56	83.33	27.78	55.56
Acinetobacter	*A. boumanni* 1, *A. calcoaceticus* 3, *A. ewoffli* 4, *A. haemolyticus* 5, *A. lwoffii* 3, *A. schindleri* 4	11	18	61.11	66.67	50.00	22.22
Erwinia	*E. amylovora 1, E. carotovora 2, E. chrysanthemi 4, E. cyperipediae 1, E. mallotivora 4*	10	12	100.00	66.67	16.67	41.67
Alcaligenes	*A. denitrificans* 4, *A. faecalis* 9	8	13	69.23	84.62	83.33	30.77
Vibrio	*V. damsela 5, V. fluvalis 1, V. metschnikovii 1, V. natriegenes 2*	7	9	11.11	11.11	0.00	0.00
Proteus	*P. mirabilis 1, P. penneri 1, P. vulgaris 2*	4	4	100.00	75.00	50.00	0.00
Edwardsiella	*E. tarda 2, E. hoshiniae 1*	3	3	100.00	0.00	0.00	0.00
Hafnia	*H. alvei* 3	3	3	100.00	66.67	66.67	33.33
Raoultella.	*R. terrigena* 3	3	3	100.00	66.67	33.33	33.33
Serratia	*S. fonticola* 1, *s. odorifera* 2	3	3	33.33	33.33	0.00	33.33
Bacillus	*B. sphaericus 3*	2	3	33.33	100.00	0.00	0.00
Citrobacter	*C. freundii 3*	2	3	0.00	66.67	33.33	66.67
Kluyvera	*K. cryocrescens* 2	2	2	100.00	100.00	50.00	50.00
Streptococcus	*S. milleri 1, S. suis 1*	2	2	100.00	100.00	50.00	0.00
Xenorhabdus	*X. luminescens* 2	2	2	50.00	100.00	100.00	50.00
Aerococcus	*Aerococcus* species 1	1	1	100.00	100.00	100.00	0.00
Flavimonas.	*F. oryzihabitans* 1	1	1	100.00	100.00	100.00	0.00
Pragia.	*P. fontium* 1	1	1	100.00	100.00	100.00	0.00
Providencia	*P. rettgeri* 1	1	1	100.00	100.00	100.00	0.00
Total bacteria of 25 genera		111	363	66.94	71.35	48.48	30.03

Table 1: Frequency of bacteria isolated from water samples from different sources in and around Bareilly, UP.

Of the 111 samples tested almost 50% {55, city drinking water (cdw) 8, village drinking water (vdw) 6, city pond water (cpw) 15, village pond water (vpw) 8, sewage water 3, swabs of water tap handles (wth) 15)} were positive for one or more types of ESKAPE bacteria. In the study, a total of 98 ESKAPE pathogen isolates were detected in water samples and on water tap handles of which 57 were resistant to one or more carbapenems and nine isolates (8 *K. pneumoniae* and one *P. aeruginosa*) produced MBL, phenotypically detected with MBL E-strips. The most common ESKAPE pathogen (Table 3) was *P. aeruginosa* (37) from 29 samples followed by *K. pneumoniae* (21) from 17 samples, *E. faecium* (19) from 9 samples, *Enterobacter* spp. (18) from 14 samples while *Acinetobacter baumannii* and *S. aureus* isolates were detected in one and two samples, respectively (Table 3).

ESKAPE Bacteria	No. of isolates	Source of isolation and number of isolates	Carbapenem resistant isolates [source and metallo-β-lactamse (MBL) producing strains]
Enterococcus faecium	19	vpw 3, cpw 16	19 [vpw 3, cpw 16]
Staphylococcus aureus	2	cpw 1, wth 1	cpw 1
Klebsiellapneumoniae	21	vdw 5, cdw 1, vpw 2, cpw 7, sewage 2, wth 4	15 [3 vdw (MBL 3), cpw 6 (MBL 2), vpw 1, sewage 1 (MBL 1), wth 3 (MBL 2)]
Acinetobacter baumannii	1	cpw 1	0
Pseudomonas aeruginosa	37	cdw 8, vdw 1, cpw 5, vpw 4, sewage 2, wth 17	18 [cdw 5, vdw 1, cpw 4, vpw 3 (MBL 1), wth 5]
Enterobacter spp.	18	vdw 2, cdw 1, cpw 8, vpw 2, wth 5	5 [cpw 4, wth 1]

Table 3: Details of ESKAPE pathogens detected in water samples from different sources.

Note: vpw, Village Pond Water; cpw, City Pond Water; vdw, Village Drinking Water; cdw, City Drinking Water; wth, Water Tap Handles.

Drug resistance among bacteria isolated from different sources varied a lot for both herbal antimicrobials (Table 4) and antibiotics (Table 5). However, there was significant positive correlation (r, 0.4, p, <0.005) between herbal antimicrobial resistance-indices (HMARI) and antibiotic resistance-indices (MARI) for bacteria isolated from water and water bodies. Similar correlation was also observed for multiple drug-resistance (MDR) and multiple herbal antimicrobial drug-resistance (MHADR; r, 0.36; p, 0.005) among bacteria isolated from water sources indicating the co-occurrence of herbal antimicrobial resistance (HAMR) and conventional antimicrobial resistance (AMR).

Sources of samples	City Pond water	Village pond water	City Drinking water	Village drinking water	Sewage water	Water tap handles	All
Number of samples tested	31	14	17	19	7	23	111
Number of isolates tested	185	64	29	31	10	44	363
Ajowan oil	12.0	12.5	50.0	16.7	50.0	8.0	14.89
Betel leaf oil	41.2	49.2	57.1	41.7	70.0	12.0	42.12
Carvacrol	8.7	9.4	64.3	33.3	50.0	4.0	13.31
Cinnamledehyde	28.3	32.8	28.6	33.3	44.4	4.0	27.92
Cinnamon oil	26.1	28.1	42.9	58.3	40.0	4.0	27.18
Citral	45.1	45.3	78.6	50.0	88.9	40.0	47.73
Guggul oil	77.2	71.9	92.9	85.0	80.0	84.0	77.92
Holy basil oil	33.7	35.9	64.3	41.7	50.0	20.0	35.28
Lemongrass oil	51.6	60.9	78.6	85.0	80.0	64.0	58.68

Marjoram essential oil	66.7	76.6	78.6	33.3	NT	68.0	68.12
Rosewood oil	64.6	70.3	64.3	16.7	NT	32.0	60.69
Sandalwood oil	83.5	72.6	92.9	70.0	80.0	88.0	81.11
Thyme oil	19.8	20.3	64.3	16.7	NT	8.0	20.89
Multiple herbal drug resistance (MHDR)	71.4	84.4	44.8	54.8	80.0	43.2	66.9
Multiple herbal antimicrobial resistance index (MHARI)*	0.422	0.450	0.659	0.624	0.625	0.335	0.451

Table 4: Herbal antimicrobial drug resistance (%) in bacteria isolated from different water samples.

NT, Not Tested; all values except of MHARI shown against different antimicrobials are in % of strains showing resistance, MHAR indices show the average of MHARI indices for the specific group of the isolates.

Sources of samples	City pond water	Village pond water	City drinking water	Village drinking water	Swage water	Water tap handles	All
Number of samples tested	31	14	17	19	7	23	111
Number of isolates tested	185	64	29	31	10	44	363
Amoxicillin	70.2	64.1	75.0	50.0	100.0	88.9	68.95
Amoxicillin+ clavulanic acid	55.2	50.8	42.9	30.0	90.0	NT	52.91
Ampicillin	75.5	67.2	61.9	53.8	100.0	47.4	70.20
Azithromycin	50.9	35.7	0.0	0.0	100.0	NT	44.44
Cefepime	47.3	31.3	18.2	0.0	100.0	8.0	39.41
Cefotaxime	74.3	52.6	9.5	50.0	60.0	22.2	54.79
Cefotaxime+ clavulanic acid	30.1	25.0	5.0	9.1	100.0	11.1	21.95
Cefoxitin	64.4	59.3	75.0	46.2	66.7	NT	62.34
Chloramphenicol	20.3	20.3	40.0	23.8	60.0	29.5	24.28
Ciprofloxacin	44.8	28.1	12.0	5.3	20.0	15.9	32.75
Colistin	41.5	54.3	64.3	14.3	66.7	38.7	44.53
Cotrimoxazole	35.5	31.3	39.3	10.0	88.9	38.6	35.34
Ertapenem	72.3	60.8	42.9	9.1	100.0	24.0	58.00
Erythromycin	89.6	77.8	80.3	100.0	88.9	100.0	87.40
Fosfomycin	28.6	85.7	85.7	83.3	NT	96.0	73.42
Gentamicin	36.5	29.7	12.0	3.2	40.0	7.0	27.12
Imipenem	45.8	32.8	0.0	0.0	0.0	33.3	36.68
Linezolid	2.4	29.4	NT	NT	NT	NT	10.17
Meropenem	55.6	39.7	0.0	12.5	0.0	25.0	42.34
Moxalactam	69.7	50.0	70.0	18.2	66.7	32.0	57.50
Nalidixic acid	67.7	56.4	66.7	34.8	77.8	#DIV/0!	61.27
Nitrofurantoin	42.6	39.3	42.9	30.0	66.7	59.1	43.70
Oxacillin	93.9	94.6	NT	NT	NT	NT	94.15

Piperacillin+ Taztobactam	56.8	34.4	0.0	NT	70.0	NT	50.67
Spectinomycin	47.7	49.1	NT	NT	NT	NT	48.17
Tetracycline	39.3	40.0	40.0	45.2	60.0	25.6	38.87
Tigecycline	17.3	0.0	44.0	23.1	80.0	11.4	24.07
Vancomycin	32.7	61.1	NT	NT	100.0	NT	41.18
ESBL positive	24.3	25.0	51.7	45.2	50.0	31.8	30.0
Multiple drug resistant	77.3	82.8	51.7	38.7	100.0	59.1	71.3
Multiple antimicrobial resistance index (MARI)	0.635	0.615	0.373	0.238	0.634	0.311	0.537
Carbapenem resistant	60.0	56.3	20.7	29.0	10.0	29.5	47.7

Table 5: Antimicrobial drug resistance (%) in bacteria isolated from different water samples.

NT, Not Tested; ESBL, phenotypically positive for extended spectrum β-lactamase production; all values except of MARI shown against different antimicrobials are in % of strains showing resistance, MAR indices show the average of MHARI indices for the specific group of the isolates.

Among all the 363 isolates of bacteria, 71.3% isolates had MDR, however, all isolates from sewage were of MDR type. In total, only 30% isolates produced ESBL but it varied (Tab. 5) for isolates from different sources, more than half of the bacteria isolated from drinking water in Bareilly city (51.7%) and about one forth of the isolates from city ponds (24.3%) produced ESBL. However, the most alarming was isolation of carbapenem-resistant (liable to convert a potential pathogen to a superbug) bacteria at alarmingly high rate, 47.7%. Carbapenem-resistance was more often (p, 0.01) detected in GPBs (67.9%) than in GNBs (41.6%). Carbapenem resistance was the least in isolates from sewage water and the most common among isolates from city ponds' (used for buffalo wallowing) water samples. However, there was no significant (p, >0.05) difference in rate of isolation of carbapenem-resistant GPBs or GNBs from different sources (Table 6).

Genus of bacteria	Number of CR isolates (tested)	Species of bacteria with CR (source of isolation, vpw, village pond water; cpw, city pond water; vdw, village drinking water; cdw, city drinking water; wth, water tap handles)
Acientobacter	9 (18)	*A. calcoaceticus* 1 (cpw), *A. ewoffli* 1 (cpw), *A. haemolyticus* 4 (3 vpw, 1 cpw), *A. lwoffii* 2 (cpw), *A. schindleri* 1 (vpw)
Aerococcus	1 (1)	*Aerococcus* spp. (vpw)
Aeromonas	21 (49)	*A. bestiarum* 1 (cpw), *A. hydrophila* 4 (3 cpw, 1 vpw), *A. jandaei* 5 (3 cpw, 2 vpw), *A. media* 3 (cpw), *A. salmonicida* 2 (1 vdw, 1 cpw), *A. schubertii* 5 (3 cpw, 2 vpw), A. sobria 1 (cpw)
Alcaligenes	10 (13)	*A. denitrificans* 4 (cpw), *A. faecalis* 6 (cpw)
Citrobacter	1 (3)	*C. freundii* 1 (sewage)
Enterobacter	5 (18)	*E. aerogenes* 2 (wth), *E. agglomerans* 3 (2 wth, 1 cpw)
Enterococcus	27 (39)	*E. faecalis* 4 (1 vpw, 3 cpw), *E. faecium* 19 (3 vpw, 16 cpw), *E. mundtii* 2 (1 vpw, 1 cpw), *E. pseudoavium* 1 (cpw), *E. solitarius* 1 (vpw)
Erwinia	2 (12)	*E. chrysanthemi* 2 (1 cpw, 1 vpw)
Escherichia	15 (58)	*E. coli 15* (1 wth, 12 cpw, 2 vpw)
Flavimonas	1 (1)	*F. Oryzihabitans* 1 (vpw)
Hafnia	2 (3)	*H. alvei* 2 (1 cpw, 1 vpw)

Klebsiella	15 (24)	*K. ozaenae* 2 (cpw), *K. pneumoniae* 13 (3 vdw, 1 vpw, 2, wth, 7 cpw)
Kluyvera	1 (2)	*K. cryocrescens* 1 (cpw)
Pragia	1 (1)	*P. fontium* 1 (cpw)
Proteus	2 (4)	*P. penneri* 1 (vpw), P. mirabilis 1 (cpw)
Providencia	1 (1)	*P. rettgeri* 1 (vpw)
Pseudomonas	27 (52)	*P. aeruginosa* 18 (5 cdw, 4 cpw, 5 wth, vdw 1, 3 vpw), *P. amltofila* 3 (cpw), *P. pseudoalcaligenes* 5 (1 vdw, 1 cdw, 3 cpw), *P. stutzeri* 1 (cpw)
Raoultella	1 (3)	*R. terrigena* 1 (cpw),
Staphylococcus	28 (39)	*S. arlettae 19* (4 vpw, 15 cpw), *S. aureus* 1 (cpw), *S. acpitis* ssp. *capitis* 2 (1 cpw, 1 vpw), *S. haemolyticus* 4 (1 vpw, 3 cpw), *S. kloosii* 1 (cpw), *S. sciuri* 1 (cpw)
Streptococcus	1 (2)	*S. suis* 1 (cpw)
Xenorhabdus	2 (2)	*X. Luminescens* 2 (vpw)
Total	173 (363)	Gram-negative bacteria 116 (279), Gram-positive bacteria 57 (84) Cdw (6) 29, vdw 6 (31), vpw 36 (64), cpw 111 (185), swage 1 (10), wth 13 (44)

Table 6: Carbapenem –resistant (CR) bacteria detected from different water sources.

*Source of sample had no statistically significant (p, >0.05) effect on prevalence of CR strains of bacteria in water samples.

For herbal antimicrobials, *Erwinia* spp. isolates were the most resistant ones with MHDR in 100% isolates, followed by staphylococci (87.2%) and pseudomonads (84.62%), the occurrence was significantly more common (p, ≤0.05) than in isolates of *Klebsiella* (70.83%), *enterococci* (69.23%), *Alcaligenes* spp. (69%), *Aeromonas* spp. (59.19%), *Enterobacter* spp. (55.56%) and *E. coli* (50%). None of the *Citrobacter* species isolates had MHDR and only 11% of *Vibrio* spp. isolates had MHDR. Most of the staphylococci (94.88%) isolates had MDR closely followed by isolates of *Alcaligenes* spp. (84.62%), *Klebsiella* spp. (83.33%), *Enterobacter* spp. (83.33%), *Pseudomonas* spp. (80.77), *Enterococcus* spp. (79.48%) and *Proteus* spp. (75%); and these proportions of MDR isolates were significantly (p, ≤0.05) higher than among isolates of *Aeromonas* spp. (67.35%), *Erwinia* spp. (66.67%), *Acinetobacter* spp. (66.67%), *Escherichia* spp. (44.83%), *Edwardsiella* spp. (0%) and *Vibrio* spp. (11%). Though in MDR and MHDR *Alcaligenes* spp. and *Klebsiella* spp. legged behind, in MBL production they lead the others with significantly higher (p, ≤0.05) proportions of carabapenem-resistant isolates than other common bacteria detected in water, 83.33% and 75% isolates, respectively. Isolates of *Erwinia* spp. with 100% MHDR had CR only in 16.67% isolates but 25.86% of *E. coli* isolates were resistant to carbapenems.

Among herbal antimicrobials, carvacrol (active ingredient of thyme oil, oregano oil and ajowan oil) was the best in antimicrobial activity, inhibiting 86.7% isolates, closely followed by ajowan oil (85.1%) and thyme oil (79.1%). Both, cinnamon oil and cinnamaldehyde (active ingredient of cinnamon bark), inhibited >72% isolates (Table 3). None of the other herbal antimicrobials inhibited ≥70% of the bacterial isolates and sandalwood oil was the least effective antimicrobial inhibiting only 18.9% of the isolates. Sensitivity of the bacterial isolates from different sources also varied significantly for different herbal antimicrobials. Resistance to ajowan oil, carvacrol, thyme oil and cinnamon oil was significantly (p, ≤0.05) more common among isolates from drinking water and sewage water than those from pond water or present on water faucet handles. However, isolates from pond water were more often (p, ≤0.01) resistant to betel leaf oil, rosewood oil, cinnamon oil, and cinnamldehyde than those present on water tap handles. Drinking water from city sources was more often (p, ≤ 0.02) the source of rosewood oil and marjoram oil resistant bacteria than drinking water in villages, however, it was opposite with respect to sensitivity of isolates to betel leaf oil (p, 0.05) and lemongrass oil (p, 0.02).

Over all, the most effective antimicrobial on bacterial isolates from samples taken from different water sources were linezolid (only against GPBs; 89.83%), cefotaxime+ clavulanic acid (78.05%), tigecycline (75.93%) and chloramphenicol (75.72%). However, surprisingly the high-end antibiotics including imipenem

(one of the most effective carbapenem) and colistin failed to inhibit 36.68% and 44.53% isolates, respectively, and old antimicrobials like gentamicin, ciprofloxacin and cotrimoxazole failed only in 27.1%, 32.8% and 35.3% cases, respectively. However, on further analysis it was evident that sensitivity of isolates from different sources (Tab. 5) varied significantly for different antibiotics. The isolates from village sources were more often resistant to linezolid than those from city sources. Gentamicin, cefotaxime+ clavulanic acid resistance was significantly (p, ≤0.01) more common among isolates from sewage and pond water samples than isolates from drinking water samples. In contrast, tigecycline-resistance was less rampant (p, 0.01) in bacteria from pond and sewage water samples than those from drinking water samples. Chloramphenicol-resistant isolates were most commonly isolated from sewage water than from pond (p, 0.003) and drinking (p, 0.05) water samples. Sewage water had similar probability for ciprofloxacin-resistance bacteria as the samples of drinking water and water tap handles but the isolates from pond water had significantly higher probability of being ciprofloxacin-resistant (p, ≤0.001) than bacteria from drinking water sources. In contrast, cotrimoxazole-resistance was more often detected in isolates from swage water ((p, ≤0.005) than among isolates from pond water, drinking water and water tap handles. Further, drinking water from city sources had 5.8 times higher odds (p, 0.002) to carry cotrimoxazole-resistant bacteria than drinking water from village supply. Sensitivity of bacteria to most of the herbal antimicrobials had negative correlation with MDR, CR and ESBL positivity indicating that herbal antimicrobials cannot be an alternative to treat AMR infections.

On ESBL producers, rosewood oil, tetracycline, chloramphenicol, carbapenems, amoxicillin+clavulanic acid, azithromycin, moxalactam, piperacillin+taztobactam, and spectinomycin were more promising (p, ≤0.01) than on non-ESBL isolates. Correlation analysis further revealed that on MDR and MHDR strains of GPBs, erythromycin, vancomycin and linezolid were more promising (p, ≤0.01) than on non-MDR strains. However, on MDR strains of GNBs, fosfomycin appeared to be more active than on non-MDR GNB strains (p, ≤0.01).

The linezolid-sensitivity also had negative correlation (p, ≤0.01) with sensitivity to betel leaf oil, thyme oil, cinnamon oil, holy basil oil, cinnamaldehyde, nalidixic acid, ciprofloxacin, colistin, moxalactam, fosfomycin and cefepime. Vancomycin-sensitivity of bacteria had negative correlation (p, ≤0.01) with their sensitivity to thyme oil, cinnamon oil, holy basil oil, cinnamaldehyde, nalidixic acid, ciprofloxacin, meropenem, azithromycin, colistin, cefotaxime, moxalactam and cefepime. The isolates more often sensitive to fosfomycin were less sensitivity (p, ≤0.01) to nalidixic acid, ciprofloxacin, meropenem, imipenem, azithromycin, cefotaxime, moxalactam, cefepime, piperacillin+ tazobactam, and linezolid. and cefepime. Tigecycline was more active (p, ≤0.01) on cefepime, moxalactam, meropenem and imipenem resistant isolates than on isolates sensitive to the latter group of antibiotics. In general, on carbapenem-resistant (CR) GPBs vancomycin and linezolid have better activity than on non-CR GPB strains. Though both azithromycin and erythromycin are macrolides, sensitivity of bacterial strains to azithromycin but not to erythromycin was negatively correlated with their sensitivity to vancomycin and fosphomycin.

Bacteria of the same genus isolated from different sources varied in their sensitivity to antimicrobials and had dissimilar pattern of source effect. However, 33% of drinking water samples had *E. coli*, none of the isolate was carbapenem-resistant but CR in *E. coli* of from samples of pond water and water faucet handles was significantly (p, <0.01) higher than *E. coli* from drinking water. Similarly, pond water *E. coli* were more often (p < 0.01) MDR type than those from drinking water and water faucet handles. However, ESBL production was rare among *E. coli* from pond water samples and significantly less than those isolated from water faucet handles (p, <0.01). Though MHDR was detected in *E. coli* from all sources, it was significantly more common among *E. coli* from pond water and faucet handle swab samples (p, <0.02) than among those from other sources.

Aeromonads from different sources hardly had any significant difference in their herbal antimicrobial and antibiotic sensitivity patterns with respect to MHDR, MDR, CR and ESBL production but those from drinking water were more often resistant to carvacrol, cinnamon oil, holy basil oil and LGO (p, <0.5) than those from pond water samples.

Enterobacter species isolates from tap water handles had more probability (p, <0.05) of being CR than those from drinking or pond water samples. More of the isolates from drinking water were resistant to holy basil oil and citral than isolates from pond

water (p, <0.05). In contrast to *Enterobacter*, *Enterococcus* species isolates from pond water had significantly higher probability (p, <0.05) than those from water tap handles for MHADR, MDR and resistance to carbapenems, tetracycline, gentamicin, ciprofloxacin, and cefotaxime.

Klebsiella isolates from pond, drinking or sewage water not varied much for their MHDR, MDR, CR and ESBL production. However, *Klebsiella* isolated from city ponds were significantly more often (p, <0.05) MDR type and produced ESBL than *Klebsiella* isolated from village pond water samples. *Klebsiella* from pond water and on water tap handles were more often (p, <0.02) resistant to ciprofloxacin, and imipenem but less often (p, <0.05) to carvacrol and cinnamon oil than isolates from drinking water.

Staphylococci from city pond water were more often resistant to thyme oil (p, 0.03), gentamicin (p, 0.04), imipenem (p, 0.01), cephalosporins (p, <0.05) but more often sensitive to linezolid (p, 0.03) than isolates from water samples of village ponds.

Pseudomonas species isolates from drinking water not varied significantly in their MDR, MHDR, CR and ESBL productions from those isolated from pond water or water tap handles. However, pond water samples were significantly (p, 0.02) better source of CR pseudomonads (73.7%) than water tap handles (29.4%). Pseudomonads from drinking water samples were more often (p, ≤0.03) resistant to tigecycline (100%), colistin (72.7%), carvacrol (90.9%), thyme oil (90.9%) and ajowan oil (63.6%) than those isolated from pond water and water tap handles (25.9%, 33.3%, 9.1%, 19.4% and 9.1%, respectively).

Discussion

Water may carry a variety of bacteria depending on the source or purpose of use of the water. In the present study, *Pseudomonas* spp. isolates dominated in occurrence and was detected in 39 samples followed by *Escherichia* (32), aeromonads (29), enterococci (21), klebsiellae and staphylococci (in 20 samples each). Other bacteria belonging to 19 more genera were also isolated from one or more number of samples. In earlier studies too isolation of a variety of different bacteria has been reported from drinking water, surface water, river system and other sources [12,13]. The dominance of *Pseudomonas* in water samples is reported world over [14]. Recently, many of the bacteria isolated from waterbodies have been reported to cause septicaemic infections [15], and abortions in wildlife [16], indicating the importance of keeping waterbodies free of potentially pathogenic bacteria.

In the study, water tap handles were the potent source for *Pseudomonas* spp., enterococci and coliforms. Of the 23 samples of water tap handles, 44 bacteria belonging to seven genera were identified, of which 59.1% and 29.5% were resistant to multiple drugs and carbapenems, respectively. In other studies, too, a variety of bacteria have been reported on water faucet handles [17]. Water taps or faucet handles were more likely to be contaminated than other surfaces with enterococci, staphylococci and *Acinetobacter* strains because of their higher resistance to drying than other bacteria [18]. From water faucet handles isolation of MDR bacteria with superbug qualifications might be a potential public health hazard because any one who take water from the supply has to operate the water tap handle and thus at the risk of acquiring highly resistant bacteria, though the bacteria was not in the water. The situation is of public health concern as you can treat the public water supplies but how to treat the water tap handles, and how frequent is it to be treated? Should we use hand free water faucets? It may be an option but reports are there that hand-free faucets may be even more dangerous in spreading the pathogens through water [19]. On water tap handles bacteria may be more persistent due to their presence in biofilm form, and biofilm bacteria are often reported to be more resistant than those in planktonic state. Biofilms can form on all solid surfaces and biofilm formation may be associated with an increased level of mutations activating certain genes responsible for production of virulence factors. Moreover, bacteria in biofilms can be more drug resistant than siblings growing in planktonic forms [20]. Thus, the risk is much more with biofilm bacteria on faucet handles which might be persistent as biofilm than those acquired from water from the same faucet. Water faucet handles are reported to be one of the dirtiest objects in any house hold and routine cleansing and disinfection is recommended to avoid spread of infections through touching those faucets.

Almost one third of the water samples tested had *E. coli*. The observations are in concurrence to earlier observations in India and abroad [12]. Isolation of *E. coli* and other coliforms from pond water was very much expected as all the ponds under study were used for wallowing of buffaloes and contamination with dung,

the rich source of coliforms, might be responsible for coliforms in pond water. However, isolation of coliforms and specially *E. coli* from 33% drinking water samples reflects the poor sanitary state of drinking water facility and lack of chlorination. High prevalence of diarrhoeal diseases and other waterborne infections in Uttar Pradesh reported earlier might be the reflection of poor microbiological quality of water observed in the present study [2]. Only <15% isolates of *E. coli* from drinking water had MDR and none was carbapenem resistant. However, in earlier studies [12,21] much higher rate of MDR *E. coli* in drinking water has been reported in other parts of India. Though we could not detect any carbapenem-resistant *E. coli* in drinking water samples in Bareilly, it has frequently been reported since long in other cities in drinking water suspected to be contaminated with sewage [22].

Both MDR and CR were common among *E. coli* isolated from pond water (77.8% and 51.9%) indicating the higher risk from the community water sources from where animals drink water and sometimes people swim there. Though studies on pond water rarely reported MDR bacteria, in earlier studies on river water in Pune [13], 29% isolates were reported MDR types and >45% of MDR *E. coli* were resistant to carbapenems.

Enterobacter spp., an important group of ESKAPE pathogens, were present in 15 samples (2 drinking water, 8 pond water, 1 sewage water and four water tap handles). It is a commonly reported coliform group of bacteria with pathogenic potential in water in India [12,21].

Isolation of 24 *Klebsiella* spp. (*K. pneumoniae* 21, *K. ozaenae* 2, *K. oxytoca* 1) isolates from 20 samples made it the fifth most commonly isolated bacteria in the study. Of these 82.3% had MDR and 62.5% were resistant to carbapenems. Multidrug-resistant *Klebsiella* in water has commonly been reported in water along with other coliforms in India [12]. In another city of Western Uttar Pradesh, Meerut, *Klebsiella* was detected as the 3rd most common bacteria after *P. aeruginosa* and *E. coli* in drinking water samples [23]. Though there are only few reports on carbapenem-resistant klebsiellae in water before the year 2000, now it has increasingly been reported from different parts of the world. Detection of carbapenem-resistance in klebsiellae is of high public health significance because *K. pneumoniae* is one of the most common bacteria acquired through water [14] and is member of ESKAPE group of pathogens, the infections difficult to treat if acquired. Carbapenem-resistant klebsiellae (*K. oxytoca* and *K. pneumoniae*) has been reported to cause several disease outbreaks in past [24]. However, *K. ozaenae* (a potential pathogen) isolated from two pond water samples are rare in occurrence.

Aeromonas, an important waterborne pathogen, was isolated from 29 samples (26.2%), the 3rd most common bacteria isolated after *Pseudomonas* spp. and *E. coli* in the study. However, >53% pond water samples were positive for aeromonads as observed in earlier studies [25]. In untreated drinking water, too, occurrence of *Aeromonas* has been reported to vary in water with seasons, the maximum being in summer, a little bit lower rate of occurrence in drinking water samples (13.9%) in the present study may be the timing of the study, carried out in spring [26]. Of the 49 isolates in the study, 67.3% had MDR and 42.9% had CR. Though MDR aeromonads are common in water all over the world [27], isolation of CR strains from water is rarely reported.

In the study 4.8% enterococci and 11.1% staphylococci were resistant to linezolid, the similar patterns have been reported earlier claiming about 90% staphylococci and other enterococci sensitive to linezolid [28,29]. Since its introduction linezolid is considered as drug of choice to treat infection with MRSA, VRSA and VRE [30]. However, in the study 14.3% of vancomycin-resistant staphylococci and 33.3% of vancomycin-resistant faecal streptococci (VRE) were resistant to linezolid. Further, staphylococci from village pond water were more often resistant to linezolid (29.4%) than those from other water sources. Though similar observations on water samples are rare, in recent past, non-susceptibility rates are reported to be higher in certain cases and specific geographic area or health care institution [29]. Moreover, linezolid resistance of the same *Staphylococcus* strain is also reported to vary with change of the method of sensitivity assay [28], therefore, variation in observation is not that important than presence of linezolid-resistant bacteria in community ponds.

All the pseudomonads from drinking water were resistant to tigecycline and observations are in concurrence to the earlier reports that >90% *P. aeruginosa* were resistant to tigecycline [31]. However, from other sources (pond water, swage, water tap handles) many of the pseudomonads were sensitive to tigecycline probably due to the presence of other species of *Pseudomonas* which are often reported to be more sensitive to tigecycline than *P. aeruginosa* [31].

In the study 44.2% pseudomonads were resistant to colistin and from drinking water samples the proportions of resistant isolates were still higher (72.7%). The situation seems to be alarming as colistin is considered to be a last-resort antibiotic used commonly against multidrug-resistant strains of *Pseudomonas aeruginosa*. However, in recent years emergence of adaptive colistin-resistance in pseudomonas is commonly reported [32].

In water samples of Bareilly all six members of ESKAPE pathogen (mnemonic for *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) were isolated. ESKAPE pathogens are serious threat to public health and the CDC estimated that antibiotic-resistant ESKAPE pathogens cause over 2 million illnesses and approximately 23,000 deaths per year [33]. In the study, most of the ESKAPE group isolates were MDR type and all staphylococci were resistant to methicillin. The emergence of MRSA alone leads to about 80,000 cases and 11,000 deaths due to invasive infection of MRSA [33]. All 19 isolates of *Enterococcus faecium* isolated in the study were from pond water samples and had MDR and carbapenem-resistance. In recent years, variety of diseases has been caused by *E. faecium*, causing 110,000 cases each year of urinary tract infections only [34]. Though AMR and MDR is very common in *A. baumannii* isolates, the only isolate in our study was neither CR nor MDR types but its detection in water is of serious concern as it has become increasingly prevalent in many countries in Middle East [34].

In the study, strong correlation between MHADR and MDR, and HMARI and MARI traits of bacteria isolated from different samples revealed co-existence of herbal antimicrobial and antibiotic resistance. Similar observations have also been reported earlier in bacteria causing clinical infections [35,36]. Similar to antibiotics, herbal antimicrobials also varied in their efficacy on different bacteria and observations are in concurrence to earlier studies [35]. Among all the herbal antimicrobials carvacrol (or its sources like ajowan oil, thyme oil) and cinnamaldehyde (or its source cinnamon oil) were the most effective herbal antimicrobials and have been reported similarly in earlier studies too [35].

In the study ESBL producers were more efficiently inhibited by tetracycline, chloramphenicol, carbapenems, amoxicillin+clavulanic acid, azithromycin, moxalactam, piperacillin+taztobactam, and spectinomycin and MDR strains of GNBs were more often susceptible to fosfomycin. Similar type of recommendations has recently been made after studying clinical strains of bacteria [37] and is in accordance of consensus guidelines for treatment of infections with ESBL producing bacteria. Observations indicated that AMR potential and susceptibility of bacteria associated with water environment might be similar to bacteria associated with clinical infections. In the study imipenem-resistant strains were often more susceptible to tigecycline and fosfomycin. On the basis of earlier findings for treatment of infections caused by carbapenem-resistant bacteria similar group of antibiotics are recommended [37]. The observations that ESBL production and carbapenem-resistance were positively correlated ($r, >0.1$; $p, <0.05$) indicated that treatment of infections caused by ESBL producers may not respond to carbapenems and may be associated with emergence of CR in bacteria. Similar observations have also been reported recently on bacteria associated with clinical infections recommending use of non-carbapenem drugs for treatment of infections caused by ESBL producers [38].

Carbapenem-resistant bacteria are real menace of the present era of AMR expansion. The detection of CR in almost half (47.7%) of the bacterial isolates detected in 43.24% water samples in Bareilly city indicated that we are sitting on a time bomb waiting for the right time to explode. Some of the bacteria like *E. faecium* are considered inherently resistant to carbapenems [39], in the present study all *E. faecium* isolates were resistant to imipenem but only 25% of other enterococcis were meropenem-resistant. Isolation of carbapenem-resistant members of Enterobacteriaceae (CRE) from water samples is always considered as a big public health threat [39]. In the study, of the 134 isolates of Enterobacteriaceae 36.6% were CRE having resistance to one or more of the carbapenem drugs. In the present study of the 8.3 % (3 of 37) drinking water samples carried CRE (*K. pneumoniae* ssp. *pneumoniae*) and almost >33% (12 of 36) samples had one or other type of carbapenem-resistant bacteria (CRB). Similar findings on carbapenem-resistant bacteria in drinking water from different parts of the world are reported viz., in Portugal, 0.02% to 15.9% of untreated drinking water samples were positive for CRB [39], in New Delhi, 32% drinking water samples had CRB; however, in Puducherry none of the 15 water samples tested was found positive for CRBs [40]. The variation in occurrence of CRBs in water samples may be due to several factors including sewage treatment facilities, drinking water sources, industries and industrial waste management,

antibiotic use in the community and pharmaceutical industries nearby [41]. However, detection of CRBs in drinking water is a serious issue and needs continuous water quality monitoring and regular water treatment for safety of the public.

Conclusion

The study revealed the poor microbiological quality of drinking water in Bareilly city and nearby villages. The ponds often used as community water sources for recreation and wallowing of buffaloes (major source of milk) were loaded with potentially pathogenic MDR and carbapenem-resistant bacteria. Almost two third (65.2%) of water faucet (tap) handles at public places were the source of CRBs, mostly belonging to ESKAPE group of pathogens, and 52.2% carried CRE including *Enterobacter* spp. (5), *E. coli* (4) and *K. pneumoniae* ssp. *pneumoniae* (4). The study indicated the urgent need for monitoring the community water sources for potential pathogens as waterborne infections are rampant in India including the region of the study. The monitoring of water quality may necessitate the sewage and drinking water treatment to ensure safe environment and health of people and animals living in and consuming the water.

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Conflict of Interest

None to declare.

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