



# Prevalence of Drug-Resistant Bacteria in Communal Well Water in the Villages of Allahabad District, Uttar Pradesh, India

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## Abstract

The occurrence of pathogenic microorganism and gradual rise in the antibiotic-resistant bacterial (ARB) strains are the recent causes of water-borne diseases. The objective of the study was to screen private water sources for bacterial contamination and to know the antibiotic susceptibility pattern of the common bacterial isolates. Water samples from 5 villages of Allahabad district, Uttar Pradesh, India sampled over a period of 8 months and were analyzed for their bacteriological quality. Antibigram of isolates was determined using the Kirby-Bauer disk diffusion method. Isolates were tested for their susceptibility to thirty-three commonly used antibiotics. The organisms isolated include *Pseudomonas* spp., *Klebsiella* spp., *Proteus* spp., *Enterococcus faecalis*, *Enterobacter* spp. and *Escherichia coli*. Out of 60 strains tested at pre-treatment, 24 (40%) were susceptible to all the antibiotics, 36 (60%) were resistant to at least one antibiotic and 32 (53.3%) were resistant to two or more antibiotics. Among 67 strains tested in post-treatment well water samples, 18 (26.8%) found to be susceptible to all antibiotics, 50 (74.6%) were resistant to at least one antibiotic and 47 (70.1%) found to be resistant to two or more antibiotics. Water treatment might increase the antibiotic resistance of surviving bacteria, and water distribution systems may serve as an important reservoir for the spread of antibiotic resistance to opportunistic pathogens. In the interest of public health, water supplies should be tested regularly to confirm their freedom from contamination. Routine microbiological surveillance of water sources and an effective waste management system in rural areas is required.

## Keywords

Antibiotics, Bacterial resistance, Coliform count, Double pot method, Rural area, Well water.

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## INTRODUCTION

All living organisms require water for survival and fundamental human need. Water is essential for the safeguarding of living organisms and conservation of ecosystem health. Polluted water isn't just dirty, it's deadly. Over 783 million people worldwide continued to use unimproved sources to meet their drinking water needs [1]. In India, over one lac people die of water-borne diseases annually. The country faces a huge challenge in ensuring safe water supply. Some 1.8 million people die every year of diarrheal diseases like cholera. Tens of millions of others are seriously sickened by a host of water-related ailments, many of which are easily preventable. Because of its importance to human existence and public health, much of the concern about drinking water has focused on toxic chemicals, radiological hazards microbial water quality, and the occurrence of pathogens [2-3].

Antibiotics are among the most commonly used and successful group of pharmaceuticals used for human medicine [4]. Rapid spread in resistance to these antibiotics has caused medical concerns to both public and health professionals.<sup>5</sup> Many factors affect the source and fate of these bacteria. Most common reason for development of resistance is inappropriate use of antibiotics such as not finishing a prescription or over-use of the drugs [5]. Moreover, antibiotic resistance is naturally present in the environment, since most antibiotics are produced by microorganisms [6]. The spread of antibiotic resistance bacteria and antibiotic resistance genes in the environment has been the subject of growing concern and is reported almost daily in both popular and scientific literatures [7]. The presence of antibiotic resistance genes has been confirmed in different environments being recognized as a new emerging contaminant in the environment [8].

Presence of antibiotic resistance bacteria in drinking water was first time reported in 1981 [9]. Wealth of recent studies are also reporting the antibiotic resistant bacteria and genes in drinking water [10-12]. Earlier it was proposed that antibiotic resistance transfer from commensal enteric bacteria to pathogenic enteric bacteria and vice versa [13-15]. Such organisms, with multiple resistances to antibiotics, have been isolated in appreciable numbers from rivers and other drinking water sources [16-17]. This is a potential risk to living beings as it is seen that there is potential direct transmission of antibiotic resistant pathogens to humans that may decrease the efficiency of the antibiotic therapy. Further, enhanced transmission of antibiotic resistance genes to commensal or

opportunistic bacteria makes drinking water an important source of antibiotic resistance [18]. Incomplete metabolism in humans and improper disposal of antibiotics to sewage treatment plants has been a main source of antibiotic release into the environment [19]. Over the time bacteria shield themselves by altering their genes and cellular mechanisms which favors their growth and reproduction [20]. The presence of trace levels of antibiotics, ARB and ARGs in source and finished water may also greatly affect public health and this is an emerging problem for the drinking water industry [9,18]. This raises the danger that people will be exposed to disease-causing bacteria that cannot be treated by antibiotics.

Bacteria carrying a gene that confers resistance to a major class of antibiotics have shown up in samples of drinking water and sewage seepage from New Delhi [21]. This alarms the need for testing drinking water sources in India. Although many reports were available on the presence of ARB and ARGs in many parts of India there was no report available on the presence of ARB and ARGs in the groundwater used for drinking purpose in Uttar Pradesh, India. In rural communities where inhabitants depend on private wells as the only source of water, the presence of antibiotic-resistant organisms could be hazardous. The present study was designed to determine the bacterial flora of communal wells pre and post treatment and the level of resistance of the enteric bacteria and other organisms from these sources to commonly used antimicrobial agents.

## MATERIALS AND METHODS

### Sample collection

A total of 30 well water samples were collected from 5 randomly selected wells. These wells are located in the Jhusi, Nibi Kala, Patel Nagar, Sonauti and Hetapatti villages of Allahabad District (Figure 1). Water from these wells is used by local community for drinking as well as household purposes. The water was collected aseptically into sterilized sampling bottles using standard water collection techniques [22]. Each well serves approx. 400 persons.

### Bacterial isolation and identification procedures

Total bacterial counts were made on standard plate count agar (Difco) incubated at 35 °C for 48-h. Multiple fermentation tube method employed to determine the presumptive coliform count (most probable number of coliforms). The procedures described by Al-Jebouri (1985)<sup>16</sup> for the isolation of *Escherichia coli* and other coliforms were used. All the samples were examined for total plate counts, as

well as faecal and total coliform counts. Colonies were sub-cultured on blood and MacConkey Agar (Difco) for purity and identification. Total 60 colonies were identified at pre-treatment and 97 colonies at post-treatment. Bacteria were identified to their genera using colonial morphology, gram reaction, and biochemical tests as described in Bergeys Manual of Systematic Bacteriology [23].

#### Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the bacterial isolates. Antibiotic multi-disks used consisted of Amoxy-clav, Cefoparazone/ sulbactam, Cefepime, Ampicillin - sulbactam, Cefuroxime, Cefixime, Ceftazidime, Gemifloxacin, Cefoparazone/ sulbactam, Nitrofurantoin, Minocycline, Cotrimoxazole, Imipenem, Moxalactam, Aztreonam, Ceftazidime/ Tazo, Tigecycline, Piperacillin - Tazobactam, Azithromycin, Amikacin, Levofloxacin, Meropenem, Colistin, Amoxycilline / sul, Moxifloxacin, Azocillin, Ceftriaxone, Cefotaxime, Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg) and Amoxicillin (30 µg). The medium used was Mueller Hinton (MH) agar. Pure cultures of organisms were enriched in nutrient broth and incubated at 37°C to a turbidity of 0.5 Macfarland standards. The MH agar was inoculated by streaking using sterile cotton swab of each of the cultures. The antibiotic disks were applied using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30 minutes to allow for diffusion of the antibiotics and the plates were incubated inverted at 37°C for 24 hours. Results were recorded by measuring the zone of inhibition and comparing with the CLSI interpretive criteria for antimicrobial disk susceptibility testing [24].

## RESULTS

### Bacterial flora of well water

The total plate counts together with the total and faecal coliform counts are given in Table 1. The total plate count values appear high for most of the wells ( $10^3$ - $10^5$  c.f.u. ml<sup>-1</sup>). Coliforms were detected in all the wells. Faecal coliform count was high in four wells and relatively low in one well (3 c.f.u./100 ml).

E. Coli were also found in the water samples from all five wells.

The different organisms isolated from all the wells are shown in Table 2. Pseudomonas spp. were the most frequently encountered, followed by Klebsiella spp. The major coliform organisms constituted 63.2% of the organism isolated at pre-treatment and 38.5% of the isolates at post-treatment.

#### Antibiotic resistance among isolates

The level of antibiotic resistance among the organism isolated before treatment of wells was high for Amoxy-clav (90%), Cefipime (56.6%), Ampicillin-sulbactam (50%), Cefoparazone/ sulbactam (38.3%) and Cefuroxime (36.6%) and resistance to Cefixime (23.3%) was less than 25%. All the isolates were resistant to Cefixime except for proteus spp (Table 3).

Level of antibiotic resistance among the organism isolated post-treatment from wells was high for Nitrofurantoin (88%), Aztreonam (41.7%), Amoxy-clav and Ampicillin-sulbactam (37.3%), Cefipime and Minocycline (35.8%) and Chloramphenicol (31.3%). Resistance to each of the other drugs was less than 30% (Table 4).

Out of 60 strains tested at pre-treatment, 24 (40%) were susceptible to all the antibiotics, 36 (60%) were resistant to at least one antibiotic and 32 (53.3%) were resistant to two or more antibiotics and among 67 strains tested in post-treatment well water samples, 18 (26.8%) found to be susceptible to all antibiotics, 50 (74.6%) were resistant to at least one antibiotic and 47 (70.1%) found to be resistant to two or more antibiotics (Table 5).

Pseudomonas and Klebsiella species exhibited 78.9% and 62.5% resistance to one or another of the antibiotics and 73.6% and 56.25% resistance to two antibiotics in pre-treatment well water samples. However, from post-treatment samples Pseudomonas showed 87.8% resistance to one or another antibiotics and 85.3% resistance to two antibiotics. Enterobacter showed equal resistance (66.6%) and Proteus (100%) to one or more antibiotics. In pre-treatment water samples, resistance for Amoxy-clav was recorded higher by the organism and in post-treatment samples, Nitrofurantoin resistance recorded to be more by the organisms isolated.

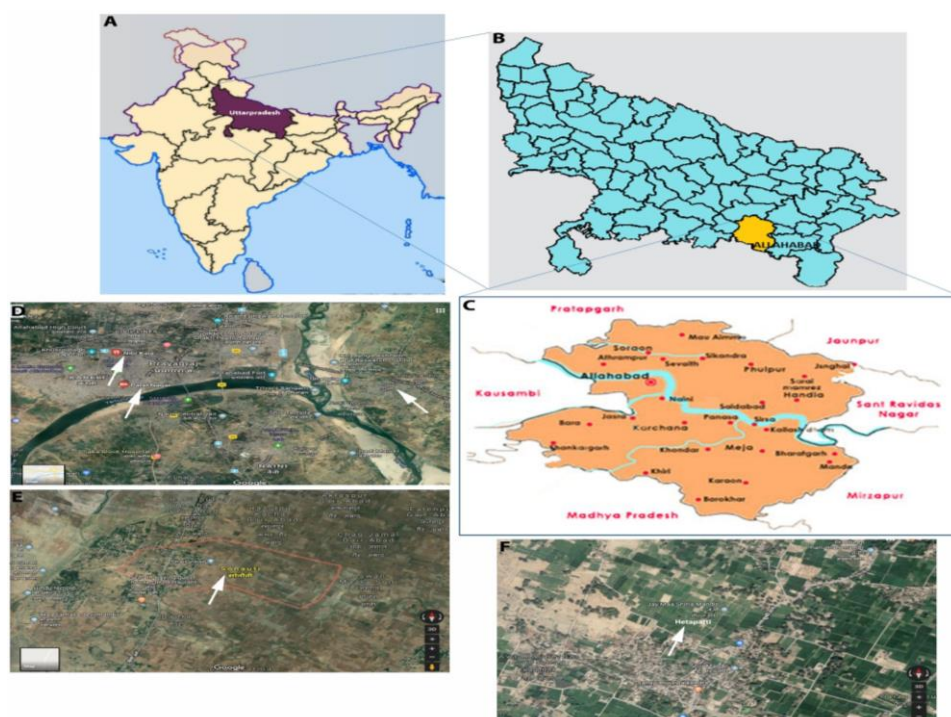
**Figure 1**


Figure 1: Represents the geographical location of villages. Water samples from open wells from these villages selected for microbiological analysis and antibiotic resistance profile. A. Map of India showing Uttar Pradesh state. B. Map of Uttar Pradesh state indicating Allahabad district. C. Map of Allahabad District. D. Satellite image of Nibi kala, Patelnagar and Jhusi villages. E. Satellite image of Sonauti village. F. Satellite image of Hetapatti village. These villages in D, E and F are shown by arrows.

**Table 1. Bacterial population in untreated well water in rural community**

Wells	SPC/ml	TC-MPN/100 ml	EC-MPN/100 ml
1	$4.2 \times 10^4$ (1.3-11.0 $\times 10^4$ )	$1.2 \times 10^3$ (2.2-2.4 $\times 10^4$ )	6 (2.0-11)
2	$4.5 \times 10^4$ (0.1-12.8 $\times 10^4$ )	2 (0-4)	4 (0-9)
3	$8.5 \times 10^4$ (1.3-4.0 $\times 10^4$ )	$7.7 \times 10^2$ (0.1-1.3 $\times 10^3$ )	5 (0-11)
4	$7.2 \times 10^4$ (5.4-9.0 $\times 10^4$ )	$2.5 \times 10^3$ (1.1-1.7 $\times 10^3$ )	4 (0-9)
5	$4.5 \times 10^3$ (2.5-6.5 $\times 10^3$ )	$5.1 \times 10^2$ (2.0-2.5 $\times 10^3$ )	2 (0-3)

Values in parentheses represent the range; SPC- Standard plate count, TC- total coliforms, EC-Escherichia coli; results are the mean values of three sets of samples collected from each of the wells

**Table 2. Range of organisms isolated from well water from rural community around Allahabad**

Organism	Pre-treatment		Post-treatment (24 hr-4week)	
	No. isolated	% occurrence	No. isolated	% occurrence
E.coli	10	16.66%	15	22.30%
Klebsiella spp.	16	26.66%	7	10.40%
Proteus spp.	3	5%	1	1.40%
Enterobacter spp.	9	15%	3	4.40%
Enterococcus spp.	3	5%	0	0%
Pseudomonas spp.	19	31.60%	41	61.10%
<b>Total</b>	<b>60</b>	<b>100%</b>	<b>67</b>	<b>100%</b>

**Table 3. Level of individual antibiotic resistance among bacteria from well water at pre-treatment**

Organism	No. of strain	AMC	C-S	FEP	SAM	CXM	CFM
E.coli	10	9	3	5	5	4	2
Klebsiella spp.	16	15	5	8	8	6	4
Proteus spp.	3	2	1	2	1	1	-
Enterobacter spp.	9	8	4	5	4	3	2
Enterococcus spp	3	2	1	2	1	1	1
Pseudomonas spp	19	18	9	12	11	7	5
Total	60	54	23	34	30	22	14
Resistance (%)		90%	38.3%	56.6%	50%	36.6%	23.3%

AMC-Amoxycilin-clavulanic acid, C-S (Cefoparazone/sulbactam), FEP- Cefipime, SAM- Ampicillin-sulbactam, CXM- Cefuroxime, CFM- Cefixime

**Table 4. Level of individual antibiotic resistance among bacteria from well water at post-treatment**

Antibiotics tested	Organism	E.coli	Klebsiella spp.	Proteus spp.	Enterobacter spp.	Enterococcus spp	Pseudomonas spp	Total	Resistance (%)
	No. of strain	15	7	1	3	0	41	67	
AMC		5	4	-	1	-	15	25	37.30%
FEP		5	8	-	-	-	11	24	35.80%
SAM		5	8	-	-	-	12	25	37.30%
CXM		4	6	-	-	-	4	14	20.80%
IPM		3	1	-	-	-	5	9	13.40%
CAZ		4	2	-	-	-	10	16	23.80%
NIT		14	4	1	1	-	39	59	88%
MIN		7	2	-	-	-	15	24	35.80%
SMX		8	3	-	-	-	15	26	38.80%
CFM		2	4	1	2	-	5	14	20.80%
MOX		4	1	-	-	-	14	19	28.30%
CHL		4	2	-	-	-	15	21	31.30%
GEM		2	1	-	-	-	1	4	5.90%
ATM		8	2	-	-	-	18	28	41.70%

AMC-Amoxycilin-clavulanic acid, FEP- Cefipime, SAM- Ampicillin-sulbactam, CXM- Cefuroxime, IPM- Imipenem, CAZ- Ceftazidime, NIT- Nitrofurantoin, MIN- Minocycline, SMX- Cotrimoxazole, CFM- Cefixime, MOX- Moxifloxacin, CHL- Chloramphenicol, GEM- Gemifloxacin, ATM- Aztreonam

**Table 5: Occurrence of antibiotic resistance among bacteria isolated from well water samples from rural communities around Allahabad**

Organisms	E. coli	Klebsiella spp.	Proteus spp.	Enterobacter spp.	Enterococcus spp	Pseudomonas spp	Total
No. susceptible No. of isolates	10	16	3	9	3	19	60
	15	7	1	3	0	41	67
	5	6	2	5	2	4	24 (40%)
	7	4	1	1	-	5	18 (26.8%)
	5 (50%)	10 (62.5)	1 (33.3%)	4 (44.4%)	1 (33.3%)	15 (78.9%)	36 (60%)
	8 (53%)	3 (42.8%)	1 (100%)	2 (66.6%)	-	36 (87.8%)	50 (74.6%)
No. (%) resistant to two	4 (40%)	9 (56.25%)	1 (33.3%)	3 (33.3%)	1 (33.3%)	14 (73.6%)	32 (53.3%)
	6 (40%)	3 (42.8%)	1 (100%)	2 (66.6%)	-	35 (85.3%)	47 (70.1%)

## DISCUSSION

Majority of villages in Uttar Pradesh, India are using underground water for daily needs, including drinking. They use water from open/unprotected, shallow hand-dug wells which often lacks hygienic conditions nearby. It is commonly surveyed and observed that well water may represent an important source of transmission of bacteria to humans. Microbiological analysis showed that wells not only contain potential pathogenic bacteria but also developing range of antibiotic resistant microbes that can have an aggravated potential to cause disease. Therefore, this study aimed to identify number of antibiotic resistant microbes in the wells of villages from Uttar Pradesh.

We selected five wells and studied the bacterial flora in the water samples where we detected approximately  $10^3$ - $10^5$  c.f.u. per mL of water. In general water samples showed presence of coliforms and fecal coliforms at high level. It is known that E. coli is rarely found in soil, vegetation or water in the absence of excremental contamination [25]. However, we observed contamination of water sample with E. coli. The wells from which E. coli were recovered in the present study could probably therefore have been exposed to some kind of fecal contamination. The bacteriological quality criteria

generally applicable to drinking water supplies to small communities are less than 10 coliforms/100 ml, and less than 2.5 E. coli per 100 ml of water [26]. Based on these none of the wells meet the international standards for potable water. Well water samples analyzed from the rural community may not therefore suitable for human consumption, without treatment. Well treatment with double pot method reduced the level of these microbes and effect was seen up to three weeks. This suggest that well water treatment is effective in reducing microbial contamination in well water, however, it required frequent check-ups, might be at every three weeks.

On characterization of total coliforms in the water samples, we found 60 isolates at the pre-treatment level and 67 at post-treatment. These isolates consisted of E. coli, Klebsiella spp., Proteus spp., Enterobacter spp., Enterococcus spp., Pseudomonas spp. While Klebsiella, Proteus, Enterobacter, Enterococcus reduced at post-treatment, E. coli (16.6% to 22.3%) and Pseudomonas (31.6% to 61.15) were found to be significantly increased. Enterococcus were not present in the post-treatment water samples.

A number of previous studies have reported that antibiotic resistance bacteria are common in drinking



water [27-28]. We added to these studies by testing water both before and after treatment with range of antibiotics. We selected to test these antibiotics since these are most frequently prescribed antibiotics for the treatment of microbial infections in selected geographical area. We tested all the isolates of observed microbial species. Amoxycillin-clavulanic acid resistance was expressed by highest 54 isolates out of 60. Microbial resistance was also observed for cefipime (34 isolates, 56.6%), Ampicillin - sulbactam (30 isolates, 50%), Cefoparazone/sulbactam (23 isolates, 38.3%), Cefuroxime (22 isolates, 36.6%). Resistance to cefixime treatment was revealed by only 14 isolates (23.3%), wherein, all the isolates were resistant to Cefixime except for proteus spp. Out of 60 strains tested at pre-treatment, 24 (40%) were susceptible to all the antibiotics, 36 (60%) were resistant to at least one antibiotic and 32 (53.3%) were resistant to two or more antibiotics. Bacterial resistance was not seen for antibiotics such as Ceftazidime/ Tazo, Tigecycline, Piperacillin-Tazobactam, Moxalactam, Ciprofloxacin, Azithromycin, Amikacin, Levofloxacin, Meropenem, Colistin, Nitrofurantoin, Minocycline, Cotrimoxazole, Gemifloxacin, Cefuroxime, Imipenem, Ceftazidime, Gentamicin, Ampicillin - Sulbactam, Cefepime, Amoxycilline/ sul, Cefixime, Cefoparazone/Sulbactam, Amoxy-clav.

In post-treatment water samples, we observed 67 isolates of same bacterial strains. We further tested their resistance pattern to antibiotics. Results showed that highest number of isolates (59) were resistant for nitrofurantoin (88%). It is followed by Aztreonam (28 isolates, 41.7%), Amoxy-clav, Ampicillin-sulbactam (both 25 isolates, 37.3%), Cefipime, Minocycline (both 24 isolates 35.8%) and Chloramphenicol (21 isolates, 31.3%). Less than 30% isolates showed resistance to CXM, IPM, CAZ, CFM, MOX. Lowest number of isolates (4) showed resistance to GEM. The level of susceptibility to gentamicin observed among the isolates tested in the present study (94.1%) is comparable to the results of previous investigators [29-30]. This may in part be because Staphylococcus spp. and the Enterobacteriaceae are generally susceptible to gentamicin [31-33]. It may also be because the drug is not used as frequently as other chemotherapeutic agents on account of its nephrotoxic side effects [34]. Very few microbes showed resistance to imipenem, a broad spectrum carbapenem antibiotic (only 13.4 %) which is in close agreement with previous reports that gentamicin is more effective against the coliforms and other gram-negative bacteria [32]. Enterococcus spp did not show resistance to any of

the tested antibiotics. Most of the isolates (88%), especially the coliforms were resistant to nitrofurantoin, a sulphonamide. This is similar to the level of resistance among E. coli (94%) observed by Linton and group [14]. This is interesting observation particularly when the level of resistance among E. coli and other coliforms in the present study is considered separately. The occurrence of multiple resistant strains of Pseudomonas spp., among other species in the water samples showed comparable findings as previously reported [9,35].

Among 67 strains tested in post-treatment well water samples, 18 (26.8%) found to be susceptible to all antibiotics. It is well proved that bacteria act as a reservoir of plasmids coding for antibiotic resistance. The ingestion of such resistant bacteria by human population could lead to a transfer of drug resistance to the recipient's gut flora and/or to susceptible pathogens by cross infection [36]. The existence of multi-drug resistance among organisms in the wells studied, therefore, constitutes a public health hazard. Fifty strains (74.6%) were resistant to at least one antibiotic and 47 (70.1%) found to be resistant to two or more antibiotics. In addition, none of the isolates reveal resistance to Ceftazidime/ Tazo, Tigecycline, Piperacillin-Tazobactam, Ciprofloxacin, Azithromycin, Amikacin, Levofloxacin, Meropenem, Colistin, Gemifloxacin, Gentamicin, Amoxycilline/sul, Moxifloxacin (MXF), Azlocillin, Ceftriaxone, Cefotaxime, Sparfloxacin, Ampicillin-Sulbactam, Imipenem, Cefoparazone/ sulbactam, Moxalactam, Ciprofloxacin, Azithromycin, Amikacin, Levofloxacin, Nitrofurantoin, Cefixime, Minocycline, Cefuroxime, Cotrimoxazole, Ceftazidime.

On the whole, organisms tested in pre-treatment well water samples showed 60 % of the isolates were resistant to at least one antibiotic while 53.3 % were resistant to two or more and in post-treatment well water samples, 74.6% of the organisms tested were resistant to at least one antibiotic while 70.1 % were resistant to two or more. Such levels of resistance would be accountable, at least in part, to the uncontrolled use of antibiotics. While the use of antibiotics may not cause bacteria to become resistant, the more use of drugs can provide an intense selection pressure in favor of organisms that possess genes coding for drug resistance.

Comparison of bacterial resistance in pre-treatment and post-treatment well water sample showed that the level of resistance was high for amoxy-clav (amoxicillin/clavulanic acid combination) in pre-treatment but not in post-treatment well water samples. On the other hand, resistance was high for nitrofurantoin post-treatment sample. This suggest

that water treatment might increase the antibiotic resistance of surviving bacteria to other antibiotics, and water distribution systems may serve as an important reservoir for the spread of antibiotic resistance to opportunistic pathogens. The results clearly show that there was no drug, among those tested, to which all isolates were susceptible in both pre and post-treatment well water samples.

## CONCLUSION

It is observed that the well water used for drinking and other domestic purposes was unfit for human consumption. Additionally, well water confirmed to be a potential source of antibiotic resistant organisms. These bacteria can serve as a reservoir for antibiotic resistance genes that can enter, adapt and thrive in the ecosystem. We suggest a need of proper bacteriological surveillance in these water sources.

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## CONFLICT OF INTEREST

None declared

## ETHICAL APPROVAL

The study was approved by Institutional Ethics Committee.

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