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Isolation, Biochemical Characterization, Antibiotics Susceptibility of Enterobacteriaceae in Potable Water Sample and Primer Designing for their Rapid Detection

Juhi Saxena[1*], Sonia Siwal[1], Mratunjay Pathak[1], Saksham Gupta[1], Aparna Datta[1]

1. Dr. B. Lal Institute of Biotechnology, Malviya Industrial Area Jaipur, India

ABSTRACT

The contamination of processed or unprocessed drinking water by pathogenic bacteria has been reported worldwide. Even though a high incidence of waterborne diseases have been well documented, enterobacteriaceae is an under acknowledged pathogen of concern to public health in India. The study was aimed to isolate and biochemically characterize Enterobacteriaceae as well as to determine antibiotic sensitivity pattern and primer designing specific to their signature gene sequences for rapid detection by PCR methods. Total plate count was determined in potable water sample collected from three different sites of Jaipur city and was found to be nearly equal or more than 10^5 cfu/ ml. Enterobacteriaceae were enumerated using the most probable number method (MPN index). Enterobacteriaceae (n = 36), randomly isolated from three sites, were identified, biochemically characterized and were screened for sensitivity to 9 antimicrobials by the disk diffusion method. The isolated bacteria had shown variable susceptibility and resistance patterns in response to different antibiotics used in the study. Polymerase chain reaction based methods can be a powerful molecular technique for rapid, sensitive and specific detection. Hence, in the present study primers were also designed specific to virulence signature sequences and their specificity was assessed by BLAST.

Keywords: Antimicrobial Susceptibility, Resistance, Enterobacteriaceae, Potable water, Detection

*Corresponding Author Email: juhi.bibt@gmail.com

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INTRODUCTION

Water is the most widespread natural resource. On a global scale, pathogenic contamination of drinking water with pathogenic microbes poses the most significant health risk to humans. Numbers of disease outbreaks and poisonings result from exposure to polluted drinking water.[1]In developing countries like India, Bangladesh and Pakistan, a large population depends on untreated surface water from rivers, lakes, wells and other water resources for drinking, bathing and other domestic purposes.[2]Microbiological studies of surface water used for drinking have revealed a high bacteria count of *Salmonella* species and *E. coli*,members of the family enterobacteriaceaeas common pathogens which indicate fecal contamination of water sources. Freshwater reservoirs can be impacted by several hazardous substances through inputs from agricultural activity, sewage discharges, groundwater leaching and runoff. Filtration and chlorination are conventionally used to process as drinking water otherwise these resources would be emerged as reservoirs of fecal coliforms exhibiting resistance to multiple antimicrobials agents resulting from the addition of municipal sewage and wastes from animal production industries and hospitals.[3,4] Outbreaks of disease attributable to drinking water in susceptible and immune compromised populations lead to serious and incurable health problems. However, waterborne disease outbreaks have been associated with insufficient or irregular treatment. [5]

Isolation of pathogens from water sources is a serious public health risk and this risk is further exacerbated by the widely reported cases of resistance of enteric bacterial pathogens to several antibiotics. The overuse of antibiotics in human medicine and agriculture is a growing concern for public health. Overuse combined with inadequate wastewater treatment has led to the presence of antibiotic resistant bacteria and genes encoding antibiotic resistance in surface waters, river sediments, and the feces of wild animals exposed to waste residuals. The multiple resistance of bacteria against antibiotics resulted from the fact that domestic and industrial waste were threw away without clarifying and so affect the underground water resources. Exposure to antibiotic resistant bacteria and the corresponding antibiotic resistance genes (ARG) in surface water can occur when the waters are used for drinking. Persons having compromised immunity can have severe health complications when consumed water contaminated with antibiotic resistant bacteria. Therefore, the water quality assessment is very important for implementation of the monitoring and remediation programs to minimize the risk promoted by hazardous substances in aquatic ecosystems.[6] While much effort has been directed toward management and monitoring of antibiotic use and the prevalence of bacterial resistance to antibiotics.[7]

Polymerase Chain Reaction (PCR) based methods with the use of specific primers as compared to Conventional methods (biochemical and microscopic) for identification of pathogenic bacteria are more specific, rapid and sensitive.[8] Therefore in the present study water sample is screened for bacterial (Enterobacteriaceae) contamination and their antibiotic susceptibility. Also specific primers were designed against virulence gene sequences which can be used for the rapid detection of bacteria by PCR methods.

MATERIALS AND METHODS

Sample collection

The total study area is comprised of three sampling stations covering different areas of Jaipur city with maximum public activity (Table 1). During sample collection all the necessary precautions were taken to collect undisturbed drinking water samples. Samples were collected in pre-sterilized bottles. The experiments were performed within 6 hours of collection.[9]

Most Probable Number

To assess water quality, samples were analyzed for total coliforms (TC) using a multiple-tube fermentation technique based on lactose fermentation with production of acid and gas within 24-48 hours in a lauryl tryptose broth. Confirmed test involving inoculation into brilliant green lactose bile broth for total coliforms was required if the water sample yielded presumptively positive results. Furthermore, complete test was done inoculating EMB plates with positive tubes of confirmed test. Presence of coliform bacteria is an indication that disease-causing bacteria may be present and that water is unsafe for drinking.[10]

Enumeration of bacteria (Total Plate count)

One milliliter of water sample was serially diluted in normal saline (0.9%) in dilutionfollowed by spreading on Nutrient Agar and incubated at 37˚C for 24 hours. The colonies were counted for each dilution and total count was expressed as no. of cfu/ ml of water samples.

Characterizations of isolates

The isolates were selected based on difference in morphology and they are characterized morphologically (Gram's, endospore and negative stain), and biochemically [IMViC (Indole, Methyl Red, VogesProskauer and Citrate Utilization), Sugar fermentation, Triple sugar iron agar (TSI), Catalase, Urease, Oxidase, Coagulase and Starch hydrolysis].[11] Isolates were maintained at -20^{0}C in LB broth supplemented with 15% (v/v) glycerol.

Determination of susceptibility to antimicrobial agents

The bacterial isolates were inoculated in nutrient broth and were kept for 18-24 hours at 37^0C. After overnight growth of bacteria in nutrient broth, 100µl of each sample was spread on Muller Hinton agar plates or with the help of a sterile cotton swab. On each plates commercially prepared discs, each of which are pre-impregnated with a standard concentration (µg per disc) of a particular antibiotic (Ampicillin, Am (10); Cephalexin, PR (30); Amoxycillin, AG (30); Cefotaxime, CF (30); Doxycycline, DX (30); Amikacin, AK (30); Netilmicin, NT (30); Ofloxacin, ZN (5); Ciprofloxacin, RC (5)) are then evenly kept and lightly pressed onto the agar surface followed by incubation at 37^0C for 24 hours.

The growth of bacteria around each disc was observed after an overnight incubation and the zone of inhibition was measured using scale in millimeters with the help of standard zone scale. In this study, data for antimicrobial agent resistance of each bacterial isolate has been reported as resistant (R), isolates with reduced susceptibility (RS or intermediates) or sensitive (S) based on Clinical and Laboratory Standards Institute (CLSI) break points.[12]

Selection &Retreival of gene sequence of interest for selected bacteria

Gram negative strains (*E. coli*, *Klebsiella sp.* and *Salmonella sp*) of bacteria belonging to family Enterobacteriaceae were selected due to their prevalence in screened water sample. Gene sequences of rcs A, LT1 and invA gene present in *Klebsiellasp*, *Escherichia coli* and *Salmonella sp* respectively were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/), National Centre for Biotechnology Information, Bethesda, USA.

Specificity of selected gene sequences

The specificity of selected nucleotide sequences of specific genes of selected bacteria were determined against the known microbial genome and sequences by BLAST (Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi), programme of NCBI to ensure no homology found in other genera or species.

Primer designing

The conserved regions, unique to a target pathogen, obtained from ClustalW were used to design primers using dedicated web based software Primer3 (http://frodo.wi.mit.edu/). The primer designing parameters such as melting temperature, GC content, amplicon length, etc were taken into consideration.

Specificity of designed primers

The specificity of computed primers of selected genes were determined against the known microbial genomes and sequences by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi)programme of NCBI to ensure no homology found in other genera or species .

RESULTS AND DISCUSSION

Quantitative enumeration of total bacteria and coliform populations

It was found that all the samples from selected sites exhibited bacterial contamination**.** The standard plate count was nearly or more than 10^5 cfu/ml of water sample collected from different sites (Table 1). All the selected sites show coliform contamination. Positive confirm tubes were used to determine MPN (most probable number) per 100 ml (Figure 1). The maximum total coliform was found in SAI sample (Table 1).

Table 1: Quantitative enumeration of bacteria in the water sample collected from Jaipur city along with their sites and sample codes.

S. No	Sample Area	Sample code	Total Plate count (cfu/ ml)	MPN Index/ 100 ml
1	City Hospital	SSI	$93x10^5$	79
2	Slum Area	STI	$16x10^6$	180
3	Sanganer Market	SAI	$42x10^5$	350

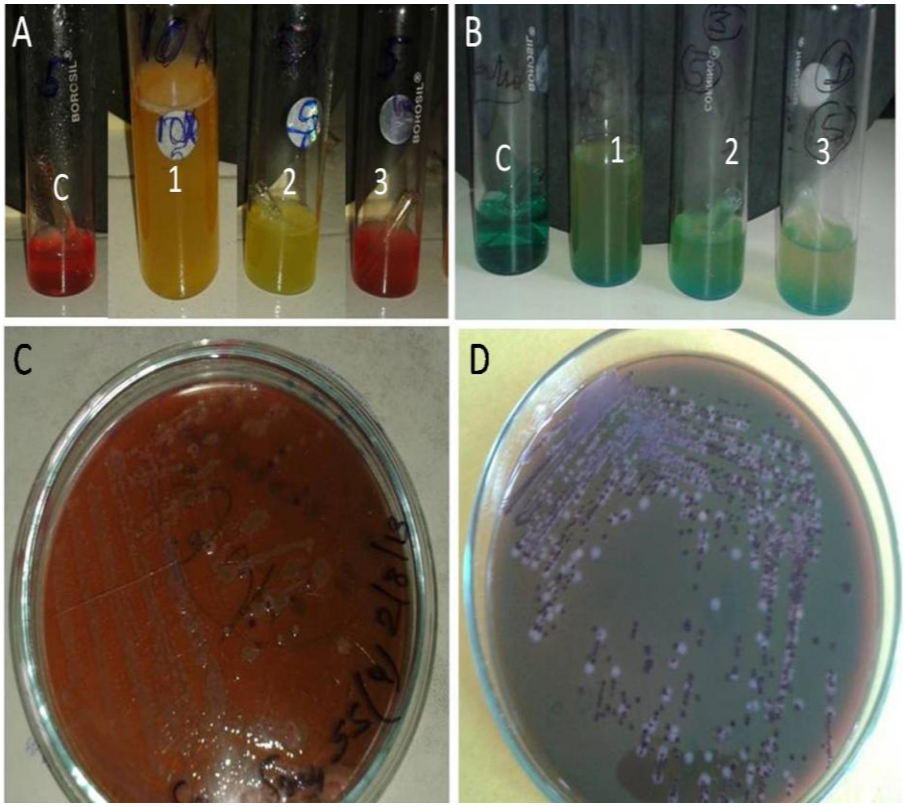


Figure 1: Multiple tube fermentation test (MPN) for coliform detection.- A – Presumptive coliform test - Positive tubes shows production of acid (colour change red to yellow) and gas production (appearance of bubble in Durham's tube) after 24 hrs of incubation. c – control, 1- LB 2X with 10 ml inoculated sample, 2 – LB 1X inoculated with 1ml sample, 3 - LB 1X inoculated with 0.1ml sample. B – Confirmed coliform test: Positive tube shows colourchange

(turbidity) and gas production in brilliant green lactose bile broth fermentation tubes. c – Control, 1, 2, 3 - BGLB tubes inoculated with 0.1 ml sample from respective positive presumptive tubes. C and D – Plate shows growth of coliform colonies on EMB agar inoculated from BGLB positive tubes.

In ideal case,the level of *E. coli* should be zero in a 100-mL sample of potable water.[13] However, in some cases it allows the presence of 10 coliforms/100 mL in drinking water.[14] In the present study, all the collected water samples exceeded the standard permissible limits.[14, 15] The presence of pathogenic microbes could be due to the defective sewage line that results into contamination of water distribution systems by fecal contaminants. Schets et al, 2005 reported the similar findings in which they found contamination of private drinking water supplies with E. coli due to defective sewage lines in Netherlands.[16]The levels of contamination clearly suggested the potential risk of water borne outbreaks.[13]

Biochemical Characterization:

The isolates were studied for their biochemical features by performing IMViC**,** TSIA test,Sugar fermentation-dextrose, lactose and sucrose, Catalase, Oxidase, and Urease. Results were compared with standard microbial chart for identification (Bergey's manual reference) and microbial species were identified (Table 2). Data suggested the contamination of *Klebsiella sp. Enterobacter sp. Proteus sp., Salmonella sp., Escherichia coli, Morganella sp. Edwardsiellasp and Shigellasp* . SSI contains 14% *Klebsiella sp.,* 51% *Escherichia coli,* 7% *Morganella sp.*7% *Proteus sp*. and 21% *Edwardsiella sp.* STI contains 25% *Klebsiella sp.,* 9% *Edwardsiella sp*., 9% *Shigella sp*., 16% *Salmonella sp*., 16% *Proteus sp*. 9% *Enterobacter sp*., 9% *Escherichia coli* and 9% *Salmonella paratyphi A*.SAI contains 50% *Escherichia coli,* 20% *Salmonella sp.,* 20% *Klebsiella and* 10%*Enterobactersp*(Figure 2). Contamination of *E. coli, Enterococcus faecalis, Klebsiellapneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa* and *Proteus mirabilis* in the surface water samples has been detected.[17] In other studies, presence of enterobacteriaceae in drinking water of South Darfur and Karachi has been reported.[18, 19]

Table 2: Identification and biochemical characterization of bacterial isolates from water sample.

Isolate	Morphology				IMViC				Sugar Fermentation			Triple Sugar Iron Agar										Strain
	Shape	Gram	Endospo	Capsule	Indole	Methyl	VogesProskauer	Citrate	Glucose	Lactose	Sucrose	Slant	Mid	Butt	H2S	Gas	Urease	Catalase	Oxidase	Coagulase	Starch HydrolysisTest	
SSI1	Rod	N	N	N	P	P	N	N	P	P	P	Y	Y	Y	N	P	N	P	N	N	N	*Escherichia coli*
SSI2	Rod	N	N	P	N	P	N	P	P	P	P	Y	Y	Y	N	P	P	P	N	N	N	*Klebsiella*sp
SSI3	Rod	N	N	N	N	P	N	P	P	P	P	Y	Y	Y	N	P	P	P	P	N	N	*Klebsiella*sp
SSI4	Rod	N	N	V	P	P	N	N	P	P	N	Y	Y	Y	N	P	N	P	P	N	N	*Escherichia. coli*
SSI5	Rod	N	N	N	P	P	N	N	P	P	N	Y	Y	Y	N	P	N	P	N	N	N	*Escherichia coli*
SSI6	Rod	N	N	N	P	P	N	N	P	P	N	P(Y)	Y	Y	N	P	N	P	N	N	N	*Escherichia. coli*
SSI7	Rod	N	N	N	P	P	N	N	P	N	P	Y	Y	Y	N	P	N	P	P	N	N	*Escherichia coli*
SSI8	Rod	N	N	P	P	P	N	N	P	N	N	P	P	P	P	P	N	P	N	N	N	*Edwardsiella*
SSI9	Rod	N	N	N	P	P	N	N	P	N	P	P(Y)	Y	Y	N	P	N	P	P	N	N	*Escherichia coli*
SSI10	Rod	N	N	N	P	P	N	N	P	N	N	P	P	P	P	P	N	P	N	N	N	*Edwardsiella*
SSI11	Rod	N	N	N	P	P	N	N	P	P	P	Y	Y	Y	N	P	N	P	N	N	N	*Escherichia coli*
SSI12	Rod	N	N	P	P	P	N	P	P	N	P	P	Y	Y	P	P	P	P	P	N	N	*Proteus vulgaris*
SSI13	Rod	N	N	N	P	P	N	N	N	N	N	P	P	P	N	P	P	P	P	N	N	*Morganellamorgani*
SSI14	Rod	N	N	N	P	P	N	N	P	N	N	P	P	P	P	P	N	P	P	N	N	*Edwardsiella*
STI1	Rod	N	N	N	N	P	N	N	P	N	P	P(Y)	Y	Y	N	p	N	P	N	N	N	*Salmonella paratyphi 'A'*
STI2	Rod	N	N	N	N	P	N	P	P	P	P	Y	Y	Y	N	p	P	P	P	N	N	*Klebsiella*sp
STI3	Rod	N	N	N	N	N	P	P	P	P	P	Y	Y	Y	N	p	N	P	N	N	N	*Enterobacter*sp
STI4	Rod	N	N	N	P	P	N	N	P	P	N	Y	Y	Y	N	p	N	P	P	N	N	*Escherichia coli*
STI5	Rod	N	N	N	N	P	N	P	P	N	N	P(Y)	Y	Y	P	N	P	P	N	N	N	*Proteus* sp

STI6	Rod	N	N	N	N	P	N	N	P	N	P	P(Y)	Y	Y	P	P	N	P	N	N	N	*Salmonella* sp
STI7	Rod	N	N	N	N	P	N	P	P	P	P	Y	Y	Y	N	P	P	P	N	N	N	*Klebsiellasp*
STI8	Rod	N	N	N	N	P	N	N	P	N	N	P	Y	Y	N	P	N	P	N	N	N	*Shigella*sp
STI9	Rod	N	N	N	P	P	N	N	P	N	N	P	P	Y	P	N	N	P	N	N	N	*Edwardsiella*
STI10	Rod	N	N	N	N	P	N	N	P	N	P	P(Y)	Y	Y	N	p	N	P	N	N	N	*Salmonella* sp
STI11	Rod	N	N	N	N	P	N	P	P	P	N	Y	Y	Y	N	p	P	P	N	N	N	*Klebsiella*sp
STI12	Rod	N	N	N	N	P	N	N	P	N	N	Y	Y	Y	P	P	P	P	P	N	N	*Proteus* sp
SAI1	Rod	N	N	N	P	P	N	N	P	P	P	Y	Y	Y	N	P	N	P	N	N	N	*Escherichia coli*
SAI2	Small rod	N	N	N	P	P	N	N	P	P	P	Y	Y	Y	N	P	N	P	N	N	N	*Escherichia coli*
SAI3	Rod	N	N	N	N	P	N	P	P	P	P	Y	Y	Y	N	P	P	P	N	N	P	*Klebsiella*sp
SAI4	Rod	N	N	N	N	P	N	P	P	P	P	Y	Y	Y	N	P	P	P	N	N	N	*Klebsiella*sp
SAI5	Small rod	N	N	N	N	N	P	P	P	P	P	Y	Y	Y	N	N	N	N	N	N	N	*Enterobacter*
SAI6	Small rod	N	N	N	P	P	N	N	P	P	P	Y	Y	Y	N	P	N	P	N	N	N	*Escherichia coli*
SAI7	Small rod	N	N	N	P	P	N	N	P	P	N	Y	Y	Y	N	P	N	P	N	N	N	*Escherichia coli*
SAI8	Rod	N	N	N	N	P	N	N	P	N	N	P(Y)	Y	Y	P	N	N	P	N	N	N	*Salmonella* sp
SAI9	Rod	N	N	N	N	P	N	N	P	N	P	P(Y)	Y	Y	N	P	N	P	N	N	N	*Salmonella* sp
SAI10	Rod	N	N	N	P	P	N	N	P	P	P	Y	Y	Y	N	P	N	P	N	N	N	*Escherichia coli*

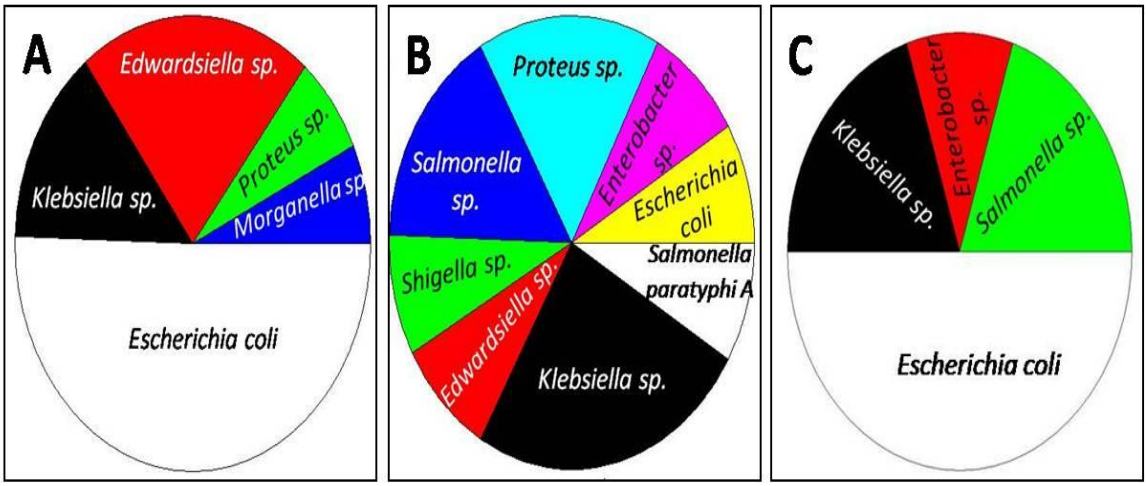


Figure 2: Percentage distribution of characterized bacteria obtained in each collected water sample. SSI contains 14% *Klebsiella*sp., 51% *Escherichia coli,* 7% *Morganella*sp.7% *Proteus* sp. and 21% *Edwardsiella*sp. STI contains 25% *Klebsiella*sp., 9% *Edwardsiella*sp., 9% *Shigella*sp., 16% *Salmonella* sp., 16% *Proteus* sp. 9% *Enterobacter*sp., 9% *Escherichia coli* and 9% *Salmonella paratyphi A.*SAI contains 50% *Escherichia coli,* 20% *Salmonella* sp., 20% *Klebsiella and* 10%*Enterobacter*sp.

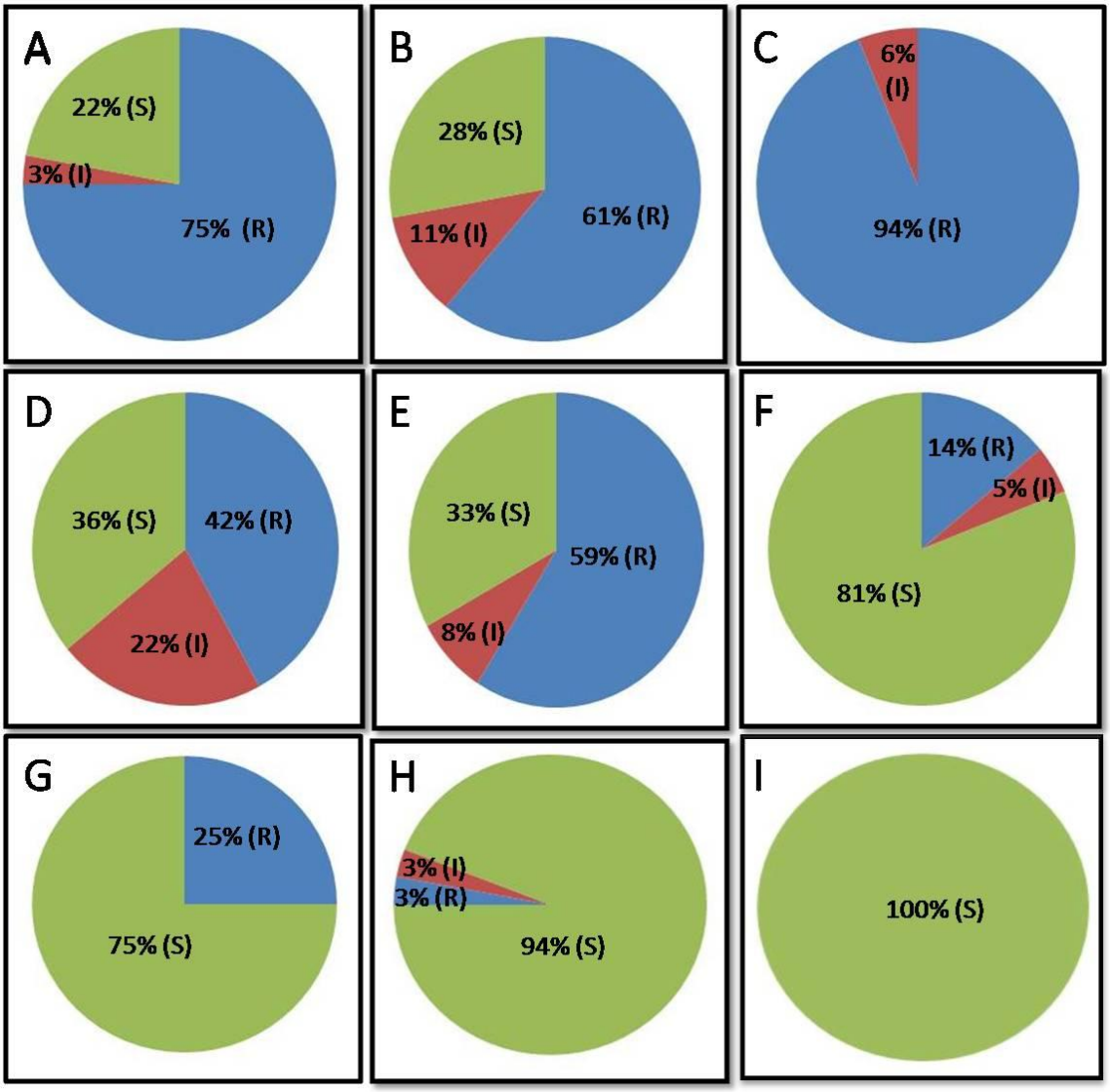
Susceptibility to antimicrobial agents:

Antibiotics are an important tool for combating infections, but bacterial strains have the ability to develop resistance against them. Antibiotic sensitivity assay of all the isolates forAmpicillin, Cefalexin, Amoxycillin, Cefotaxime, Doxycycline, Amikacin, Netilmicin, Ciprofloxacin and Ofloxacin was performed on Muller Hinton agar by disc diffusion method and zone of inhibition was measured. This zone of inhibition was compared with standard CLSI table and isolates were characterized as resistant (R), intermediate (I) and sensitive (S) **(**Table 3). The percentage of resistant (blue), reduced susceptible (red) and sensitive (green) bacterialisolates against Ampicillin (A) (R - 75%, I – 3%, S – 22%), Cephalexin (B) (R - 61%, I – 11%, S – 28%), Amoxycillin (C) (R - 94%, I – 6%), Cefotaxime (D) (R - 42%, I – 22%, S – 36%), Doxycycline (E) (R - 59%, I – 8%, S – 33%), Amikacin (F) (R - 14%, I – 5%, S – 81%), Netilmicin (G) (R - 25%, S – 75%), Ciprofloxacin (H) (R - 3%, I – 3%, S – 94%), Ofloxacin (I) (S – 100%) were obtained (Figure. 3).

Studies on antimicrobial resistance in *E. coli* isolated from humans, surface waters, cattle, and food have also been reported.[20-23] Moreover, higher antimicrobial resistance in *E. coli* isolated from urban areas than rural areas, possibly due to greater exposure to antimicrobials.[24] Earlier studies have also shown that *E. coli* isolated from clinical and surface water exhibited multimicrobial resistance.[25, 26]

Table 3: Antimicrobial Susceptibility of bacterial isolates in water sample – Sensitive (S), Resistant (R) or Intermediate (I/isolates with reduced susceptibility) to antibiotics

S.No	Strain No.	Strain Name	Zone of inhibition (mm) (R/I/S)								
			Ampicillin	Cephalexin	Amoxycillin	Cefotaxime	Doxycycline	Amikacin	Netilmicin	Ciprofloxacin	Ofloxacin
1	SSI1	*E. coli*	15/I	19/S	0/R	23/S	18/S	19/S	17/S	29/S	32/S
2	SSI2	*Klebsiella*	0/R	16/I	0/R	27/S	19/S	20/S	22/S	29/S	31/S
3	SSI3	*Klebsiella*	19/S	16/I	14/I	25/S	16/S	18/S	17/S	29/S	34/S
4	SSI4	*E. coli*	17/S	20/S	12/R	23/S	17/S	19/S	17/S	31/S	27/S
5	SSI5	*E. coli*	17/S	18/S	13/R	23/S	18/S	18/S	21/S	30/S	37/S
6	SSI6	*E. coli*	0/R	15/I	0/R	27/S	17/S	22/S	28/S	36/S	35/S
7	SSI7	*E. coli*	19/S	19/S	10/R	27/S	14/I	20/S	16/S	32/S	27/S
8	SSI8	*Edwardsiella*	20/S	18/S	10/R	25/S	17/S	22/S	15/S	32/S	33/S
9	SSI9	*E. coli*	0/R	22/S	10/R	12/R	18/s	23/S	21/S	25/S	26/S
10	SSI10	*Edwardsiella*	13/R	0/R	0/R	13/R	10/R	17/S	18/S	14/R	16/S
11	SSI11	*E. coli*	0/R	0/R	0/R	14/R	10/R	10/R	17/S	30/S	20/S
12	SSI12	*Proteus vulgaris*	17/S	14/R	14/I	14/R	16/S	22/S	22/S	30/S	28/S
13	SSI13	*Morganellam organi*	11/R	18/S	10/R	18/I	19/S	16/I	16/S	23/S	20/S
14	SSI14	*Edwardsiella*	10/R	22/S	0/R	0/R	16/S	26/S	21/S	27/S	25/S
15	STI1	*Salmonella paratyphi 'A'*	0/R	0/R	0/R	18/S	12/R	10/R	11/R	34/S	26/S
16	STI2	*Klebsiella*	0/R	0/R	0/R	16/I	10/R	23/S	12/R	38/S	36/S
17	STI3	*Enterobacter*	0/R	0/R	0/R	10/R	0/R	0/R	14/R	18/S	21/S
18	STI4	*E coli*	0/R	0/R	0/R	15/I	10/R	21/S	23/S	37/S	34/S
19	STI5	*Proteus*	0/R	0/R	0/R	16/I	0/R	27/S	23/S	35/S	31/S
20	STI6	*Salmonella*	0/R	0/R	0/R	14/R	0/R	24/S	25/S	32/S	33/S
21	STI7	*Klebsiella*	0/R	0/R	0/R	10/R	0/R	25/S	25/S	23/S	32/S
22	STI8	*Shigella*	0/R	0/R	0/R	15/I	0/R	21/S	21/S	21/S	28/S
23	STI9	*Edwardsiella*	0/R	0/R	0/R	10/R	0/R	20/S	20/S	18/S	31/S
24	STI10	*Salmonella*	0/R	0/R	0/R	17/S	0/R	21/S	21/S	20/S	31/S
25	STI11	*Klebsiella*	0/R	0/R	0/R	16/R	12/R	22/S	22/S	22/S	32/S
26	STI12	*Proteus*	11/R	21/R	0/R	13/R	14/I	27/S	27/S	28/S	30/S
27	SAI1	*E coli*	0/R	0/R	0/R	12/R	10/R	20/S	10/R	30/S	27/S
28	SAI2	*E coli*	0/R	0/R	0/R	12/R	10/R	15/I	19/S	18/I	27/S
29	SAI3	*Klebsiellasp*	0/R	0/R	0/R	12/R	10/R	17/S	0/R	30/S	27/S
30	SAI4	*Klebsiellasp*	0/R	0/R	0/R	12/R	10/R	13/R	12/R	29/S	27/S
31	SAI5	*Enterobacter*	0/R	0/R	0/R	21/I	10/R	18/S	10/R	34/S	27/S
32	SAI6	*E coli*	33/S	20/S	11/R	22/I	10/R	35/S	28/S	31/S	27/S
33	SAI7	*E coli*	10/R	10/R	10/R	23/S	14/I	10/R	10/R	26/S	27/S
34	SAI8	*Salmonella sp*	10/R	30/S	10/R	27/S	20/S	19/S	10/R	31/S	27/S
35	SAI9	*Salmonella sp*	0/R	0/R	10/R	18/I	10/R	17/S	17/S	27/S	27/S
36	SAI10	*E coli*	27/S	16/I	12/R	28/S	11/R	40/S	40/S	28/S	27/S



Computation and specificity of primers for detection of selected bacteria

Figure 3: The percentage of resistant (blue), reduced susceptible (red) and sensitive (green) bacterialisolates against Ampicillin (A) (R - 75%, I – 3%, S – 22%), Cephalexin (B) (R - 61%, I – 11%, S – 28%), Amoxycillin (C) (R - 94%, I – 6%), Cefotaxime (D) (R - 42%, I – 22%, S – 36%), Doxycycline (E) (R - 59%, I – 8%, S – 33%), Amikacin (F) (R - 14%, I – 5%, S – 81%), Netilmicin (G) (R - 25%, S – 75%), Ciprofloxacin (H) (R - 3%, I – 3%, S – 94%), Ofloxacin (I) (S – 100%)

Bacteria contain a large number of specific gene sequences which are essential for their survival and virulence (pathogenicity) of bacteria. These essential genes are more evolutionary conserved than non-essential genes in bacteria and can be used for their characterization. PCR methods being more rapid, specific, and sensitive can be used for regular monitoring and risk assessment of the microbiological quality of water sources.[27, 28]Here, pathogenic microorganism *Klebsiellasp*,

Salmonella sp andEscherichia coli belonging to family Enterobacteriaceae were selected due to their maximum prevalence in screened water sample. We have retrieved gene sequences of rcs A, LT1 and invA gene present in *Klebsiellasp*, *Escherichia coli* and *Salmonella sp* from NCBI respectively. The gene sequences were then used for primer designing unique to respective microorganism bysoftware Primer3. Results give highly specific PCR primers exhibited melting temperature 59.55^{0}C - 60.09^{0}C and amplicon size 209-249 bp(Table 4).The specificity of computed oligonucleotides (designed primer pairs) was determined against the known microbial genomes by NCBI-BLAST (Basic Local Alignment Search Tool) before selecting primer pair for PCR.Parameters such as E-values (minimum), max score (maximum) and query coverage (maximum) were analysed for the specificity of oligonucleotides, and it has been found that the computed primers were highly specific towards target sequences. Output of primer designing can be inferred for selecting primer pair for wet lab PCR assay for instance parameters such as melting temperature, GC content, amplicon length, etc were taken into consideration while selecting primer pair for PCR experiments.

Table 4:Retrieved nucleotide gene sequence with accession numbers of selected bacterial genes and designed set of Primers which can be used for PCR based detection.

Microorganisms	**Genes (Accession number)**	**Primers Sequences (5'-3')**	**Length (bp)**	**Tm (^{0}C)**	**GC (%)**	**Product size(bp)**
Klebsiella pneumonia	*rcsA*(AY059955.1)	**F:** CTATTTGCGGGTACGGAAGA	20	60.09	50.00	209
		R: TCATTTGCGTTGAGATTTGC	20	59.82	40.00	
Escherichia coli	*LT1* (AY342056)	**F:** GTTCGGAATATCGCAACACA	20	59.55	45.00	218
		R: TTTGGTCTCGGTCAGATATGC	21	60.09	47.62	
*Salmonella*sp	*invA* (EU348368)	**F:** AGTGCCGGTTTTATCGTGAC	20	60.00	50.0	249
		R: CTCGCCTTTGCTGGTTTTAG	20	60.01	50.0	

CONCLUSIONS

The present study highlights the human health risk associated with enterobacteriaceae contamination in potable water. The problem will become more severe when these pathogens harbor traits for antimicrobial resistance. Furthermore, primer designing and its validation of the

target genes prior to bench optimization of the designed PCR assay for rapid detection of enterobacteriaceae can minimize time and cost needed for in vitro verification of work. For strategic approach to protect public health there is requirement of increased surveillance for risk assessment and rapid detection methods against enterobacteriaceae.

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