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Prevalence and antimicrobial resistance patterns of diarrheagenic and uropathogenic *Escherichia coli* in Odisha, India

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ABSTRACT

Objective: The aim of this study was to determine the prevalence and antibiotic susceptibility patterns of pathogenic *Escherichia (E.) coli* causing diarrhea and urinary tract infection (UTI) in patients admitted for treatment in selected health care facilities in Odisha, an eastern Indian State.

Methods: A total of 189 rectal swabs from patients with diarrhea and 297 urine samples from patients with UTI were collected from selected health care facilities. *E. coli* isolates were phenotypically confirmed using MacConkey agar, MacConkey sorbitol agar and blood agar along with biochemical tests. These phenotypically confirmed *E. coli* isolates were investigated genotypically for known pathogenic *E. coli via* multiplex PCR. Finally, the pathogenic *E. coli* was subjected to antibiotic sensitivity test.

Results: Culture of 189 rectal swabs and 297 urine samples revealed 46 (24.3%) diarrheagenic *E. coli* (DEC) and 36 (12.1%) uropathogenic *E. coli* (UPEC). PCR analysis of DEC identified 27 (14.3%) enterotoxigenic *E. coli* (ETEC), 8 (4.2%) enteropathogenic *E. coli*, 10 (5.3%) enteroaggregative *E. coli* and 1 (0.5%) Enterohemorrhagic *E. coli* (EHEC) in diarrhea patients and 5 (1.7%) ETEC, 7 (2.4%) enteroaggregative *E. coli*, 11 (3.7%) EHEC O157:H7 and 1 (0.3%) EHEC non-O157:H7 in UTI patients. DEC and UPEC exhibited multidrug resistance with a high rate of resistance to commonly prescribed antibiotics. A high frequency of ETEC causing diarrhea, atypical enteropathogenic *E. coli* harboring the *eae* gene was found among children with acute diarrhea, and ETEC (est^+) and EHEC O157:H7 were detected in UTI patients along with high antibiotic resistance among DEC and UPEC were important findings.

Conclusions: To the best of our knowledge, this is the first study to report the prevalence of DEC and UPEC in patients admitted to hospital in Odisha, India, revealing a high rate of antimicrobial resistance.

KEYWORDS: Pathotype *Escherichia coli*; Diarrhea; Urinary tract infection (UTI); Antibiogram; Odisha

1. Introduction

Acute diarrheal disease and urinary tract infections (UTI) are major concerns, with a high morbidity and mortality in the developing world. Globally, 1.7 billion childhood diarrheal cases and 404.6 million UTI cases are registered every year causing 370 000 and 236 786 deaths, respectively[1,2]. Among the wide variety of

Significance

The study reports for the first time the prevalence and antibiotic resistance pattern among diarrheagenic *Escherichia (E.) coli* and uropathogenic *E. coli* in Odisha, India, revealing a high prevalence and antimicrobial resistance of diarrheagenic and uropathogenic *E. coli*. This will help the physicians to treat the diarrheagenic *E. coli* and uropathogenic *E. coli* associated infections appropriately.

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bacterial pathogens causing diarrhea and UTI, *Escherichia (E.) coli* is known to play a major role in intestinal and extra-intestinal infections. Out of 1.7 billion diarrheal cases globally, *E. coli* causes more than 300 million illnesses along with 200 000 deaths per year while the estimated incidence rate of *E. coli* in UTI cases occurring globally has been found to be 80%[3,4]. *E. coli* is a bacterial species that normally lives in the intestine of healthy people and animals as a commensal. But beyond this ecological niche, some strains can cause intestinal and extra-intestinal infections. In intestinal infection, a few causes mild diarrhea while some give rise to severe diarrhea with abdominal cramps, bloody diarrhea and vomiting. Diarrhea causing *E. coli* (DEC) can be divided into six pathotypes namely enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E.coli*, enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) and diffusely adherent *E. coli*[5,6]. Among EHEC, serotype O157:H7 is a major food-borne bacterial pathogen due to the production of a crucial virulence factor, verotoxin/shiga toxin[6]. Two types of verotoxins are produced by verotoxigenic *E. coli* (VTEC), namely *verotoxin1* (*vtx1*) and *verotoxin2* (*vtx2*). VTEC O157:H7 causes the majority of haemorrhagic colitis and the hemolytic uremic syndrome (HUS) globally[7]. Uropathogenic *E. coli* (UPEC) is well known as the commonest cause of UTI globally, both in the community and the hospital settings.

The rapid emergence of antimicrobial resistant strains in recent years has become a major contributing factor to treatment failure of infectious diseases like diarrhea and UTI[8]. Surveillance and monitoring of antimicrobial resistant strains have thus gained importance. This study aims to explore the prevalence and pattern of antibiotic resistance of DEC and UPEC *E. coli* so as to help the physicians to prescribe appropriate antibiotic(s) for treatment of patients in Odisha, India.

2. Materials and methods

2.1. Study settings

This was a hospital-based study conducted in Odisha, the 11th most populated state in India with a time span from February 2019 to December 2021. Patients with diarrhea admitted to the paediatric wards of Capital Hospital, Bhubaneswar, District Headquarter Hospital and PHC/CHC Hospitals of Puri District and patients with suspected UTI attending the outpatient department of SUM Hospital, Bhubaneswar, were included in our study. Informed consent was taken from patient/guardian prior to the collection of samples (stool and urine), and clinical details and demographic information were recorded in pre-structured formats.

2.2. Ethical approval

The Institutional Human Ethics Committee of ICMR Regional Medical Research Centre, Bhubaneswar (RMRCBB) approved the study with reference number ICMR-RMRCB/IHEC-2020/13 dated 16/01/2020.

2.3. Samples and data collection

2.3.1. Rectal swabs

Rectal swabs were collected from acute diarrhea patients and transported to the laboratory in sterile plastic vials containing Cary-Blair transport medium within 2 h of collection.

2.3.2. Urine samples

Freshly voided, mid-stream urine samples were collected aseptically into sterile vials from patients with symptoms suggestive of UTI. Boric acid (2.63 mg/mL) was added to prevent further bacterial multiplication and the samples were transported to RMRC (Regional Medical Research Centre) Laboratory for bacteriological analysis.

2.4. Isolation and identification of uropathogens

Urine samples were processed within 2 h of collection. Samples of 10 μL each were inoculated onto MacConkey and 5% blood agar plates and incubated at 37 ℃ for 18 h. Following overnight incubation, pure isolates of $>10^5$ cfu/mL colony counts were recorded as significant bacteriuria. Colourless and pink colonies on Sorbitol MacConkey Agar (SMAC) plates were presumed as STEC-species O157 and non-O157 respectively[9,10]. These significant isolates were then phenotypically characterized based on their cultural, morphological, and biochemical characteristics following standard taxonomic guidelines[11]. Furthermore, *E. coli* O157:H7 strains which were obtained by streaking onto SMAC plates at 37 ℃ for 16 to 18 h were genotypically confirmed to be *E. coli* O157:H7 *via* PCR method.

Template DNA was extracted from the non-sucrose fermenting colonies on SMAC by the boiling method. Multiplex PCR assay was conducted for detection of *E. coli* O157:H7 strain following the method described elsewhere[12], using the specific primers encoding the genes *vtx1*, *vtx2*, and *eaeAO157*[13,14]. PCR was performed in a

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25 μL final reaction volume, which comprised of the followings: primers for *vtx1*, *vtx2*, and *eaeA* gene (GCC Biotech PVT. Limited, India) at concentrations of 0.2, 0.2, and 0.8 mM, 10 mM dNTP (2.5 mM each of Bangalore Genei, India), 10X PCR Buffer (Bangalore Genei, India), 3 U of Taq DNA Polymerase (Bangalore Genei, India) and 3 μL of template DNA. All these components were suspended in molecular grade water (Hi-Media, India) and then subjected to PCR amplification using a thermal cycler (Hi-media, India). The cycling conditions were as follows: 94 ℃ for 2 min; 30 cycles of 94 ℃ for 30 s; 56 ℃ for 30 s; 72 ℃ for 30 s and extension at 72 ℃ for 10 min. The PCR amplicons were run on 2% agarose gel stained with ethidium bromide and visualised using a gel-documentation system (Alpha Imager, Alpha Innotech Corporation, USA).

2.5. Isolation and genetic confirmation of DEC

Rectal swabs were inoculated on thiosulphate-citrate-bile salt sucrose agar, Hektoen enteric agar, MacConkey agar and SMAC to isolate DEC and other entero-pathogens. Pink coloured bacterial isolates in MacConkey agar and colourless isolates in Sorbitol Agar were identified and DNA was isolated by boiling method using molecular grade water from the colonies obtained on original plate(s)[15]. Potential DEC were purified. After 18-24 h incubation, DNA was extracted from 10 randomly selected typical lactose fermenting colonies on MacConkey Agar and non-fermenting colonies on SMAC by boiling method. The isolated DNA were genetically confirmed as *E. coli* by performing PCR targeting the *E. coli* marker gene *alr* (Alanine Racemase)[16]. Finally, multiplex PCR assay was conducted to detect DEC: ETEC (*elt*, *est*), EPEC (*eae*, *bfpA*) and EAEC (*aatA*)[17,18] and VTEC (*vtx1*, *vtx2*, *eaeAO157*) using the specific primer pairs described elsewhere[13,14].

2.6. Antibiogram

Antimicrobial susceptibility testing of DEC and UPEC was performed on Mueller-Hinton agar as per the Clinical Laboratory Standard Institute Guidelines on a panel of antibiotics: tetracycline (TE, 30 μg), gentamicin (GEM,10 μg), streptomycin (S,10 μg), chloramphenicol (C, 30 μg), norfloxacin (NX, 10 μg), neomycin (NE 30 μg), ampicillin (AMP, 10 μg), ciprofloxacin (CIP, 15 μg), ofloxacin (OF), furazolidone (FR, 100 μg), azithromycin (ATZ, 15 μg), amikacin (AK, 30 μg) and doxycycline (DO, 30 μg)[19] .

3. Results

A total of 189 rectal swabs were collected from diarrhea patients of all age groups. Bacteriological analysis yielded *Vibrio Cholerae* O1 Ogawa 3 (1.6%), *Shigella* spp 5 (2.6%), *Salmonella* spp 1 (0.5%) and 128 *E. coli* isolates. Out of the 128 *E. coli* identified by conventional methods, 87 were confirmed as *E. coli* by detection of the *alr* gene encoding a surface antigen (Figure 1). Multiplex PCR identified 46 (24.3%) DEC including ETEC 27 (14.3%), EPEC 8 (4.2%), EAEC 10 (5.3%) and EHEC 1 (0.5%) (Table 1). Among 27 ETEC isolates, 5 (2.5%) strains were elt^+, 12 (6.3%) est^+ and 10 (5.3%) est^++elt^+ (Figure 2). Only one *E. coli* strain was recognised as O157:H7 harbouring *eaeAO157*. Among 8 EPEC isolates, 4 (2.1%) were positive for *eaeA* (aEPEC) and 2 (1.1%) positive for *eaeA*+*bfpA* (tEPEC) and 2 (1.1%) were positive for $bfpA^+$. All the 10 (5.3%) EAEC isolated strains were positive for the *aatA* gene. Among mixed infections with more than one DEC, the combination of ETEC+EAEC was most common with 6 (3.2%) followed by ETEC+EPEC 3 (1.6%), EPEC+EAEC 2 (1.1%) and ETEC+EAEC 1 (0.5%).

Table 1. Prevalence of toxigenic *Escherichia coli* with their viruence genes and other etiological bacterial pathogens associated with hospitalized acute diarrhea patients.

DEC	All *n* = 189	<1 yr *n* = 31	1-5 yr *n* = 69	5-10 yr *n* = 28	>10 yr *n* = 61
V. cholera O1	3 (1.6)	1 (3.2)	-	-	2 (3.3)
Shigella spp.	5 (2.6)	1 (3.2)	2 (2.9)	2 (7.1)	-
Salmonella spp.	1 (0.5)	-	1 (1.4)	-	-
ETEC	27 (14.3)	3 (9.7)	8 (11.6)	1(3.6)	15 (24.5)
ETEC (elt^+)	5 (2.6)	2 (6.4)	-	-	3 (5.0)
ETEC est^+	12 (6.3)	1 (3.2)	5 (7.2)	-	6 (9.8)
ETEC *est+elt*	10 (5.3)	-	3 (4.3)	-	7 (11.5)
EPEC	8 (4.2)	2 (6.4)	2 (2.9)		4 (6.6)
EPEC *eaeA*	4 (2.1)	-	-	-	4 (6.6)
EPEC $bfpA^+$	2 (1.1)	-	2 (2.9)	-	-
EPEC *eae+bfpA*	2 (1.1)	2 (6.4)	-	-	-
EAEC *aatA*	10 (5.3)	2 (6.4)	4 (5.8)	-	4 (6.6)
EHEC O157:h7 $eaeAO157^+$	1 (0.5)	-	12 (1.4)	-	
Mixed infection					
ETEC+EAEC *elt, aatA*	6 (3.2)	2 (6.5)	-	-	4 (6.6)
ETEC+EAEC *elt+est, aatA*	1(0.5)	-	-	-	1(1.6)
ETEC+EPEC *eae, est+elt*	3 (1.6)	-	-	-	3(5.0)
EPEC+EAEC *eae, aatA*	2 (1.1)	-	-	-	2(3.3)

Note: Data were expressed as *n* (%); ETEC= Enterotoxigenic *E. coli*, EPEC= Enteropathogenic *E. coli*, EAEC= Enteroaggregative *E. coli*, EHEC= Enterohemorrhagic *E. coli.*

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A total of 297 urine samples were collected from clinically significant UTI patients of various ages and sexes. Total patients with confirmed case of UTI were 197 (66.3%). Bacteriological analysis detected 120 uropathogens, *E. coli* 36 (12.1%) as the predominant pathogen followed by *Klebsiella* spp. 30 (10.1%), *Proteus* spp. 8 (2.7%), *Enterobacter* spp. 7 (2.4%), *Moragnella* spp. 5 (1.7%), *Serratia* spp. 6 (2.0%), other gram-negative bacilli 21 (7.0%) and nonspecific bacteria 20 (6.7%) (Table 2). Multiplex PCR analysis of the 36 UPEC strains exhibited that among 11 EHEC isolates 9(3.0%) harbouring *eaeAO157*, 1 *eaeAO157+vtx2* and 1 *vtx1* followed by EAEC 7 possessing *aatA* and ETEC 5 strains carrying 1 *elt*, 3 *est* and 1 *elt+est* genes. There was no significant difference in virulence genes for DEC and UPEC isolates except for *sfa* gene (P=0.012)[20].

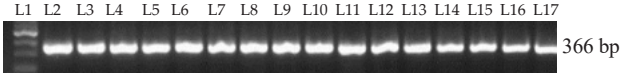

Figure 1. Simplex PCR assay for detection of *Escherichia coli* marker gene *alr* (alanine racemase). L1= 100 bp DNA Ladder, L2 to L17 are all *alr* positive *E. coli* isolates.

Table 2. Prevalence of different uropathogens among UTI Patients.

Uropathogens detected	Total urine samples collected n = 297
ETEC	5 (1.7)
est	3 (1.0)
elt	1 (0.3)
elt+est	1 (0.3)
EHEC	11 (3.7)
eaeAO157	9 (3.0)
vtx1	1 (0.3)
eaeAO157 + vtx2	1 (0.3)
EAEC	7 (2.4)
Proteus spp.	8 (2.7)
Klebsiella spp.	30 (10.1)
Enterobacter spp.	7 (2.4)
Moragnella spp.	5 (1.7)
Serratia	6 (2.0)
Gram negative *bacilli*	21 (7.0)
Non-specific bacterium	20 (6.7)

Note: Data were expressed as n (%); ETEC= Enterotoxigenic *E. coli*; EHEC= Enterohemorrhagic *E. coli*; EAEC= Enteroaggregative *E. coli.*

Antibiotic susceptibility results demonstrated that about more than 70% of DEC were resistant to ampicillin, azithromycin, norfloxacin, ciprofloxacin, ofloxacin, doxycycline and neomycin (Figure 3). A lower prevalence (<30%) of resistance was observed for chloramphenicol and gentamicin. ETEC was encountered with higher level of resistance to ampicillin, norfloxacin azithromycin, ofloxacin and neomycin while EPEC was resistant to most of the drugs except chloramphenicol and gentamycin.

UPEC isolates demonstrated higher level of resistance to commonly used antibiotics including azithromycin 70.0%, ciprofloxacin 70.0%, ofloxacin 83.0% and ampicillin 28.0%, norfloxacin 60.0% and doxycycline 60.0% (Figure 3 and 4). Similar to DEC, UPEC expressed high susceptibility to gentamicin 86.0% and chloramphenicol 73.0%.

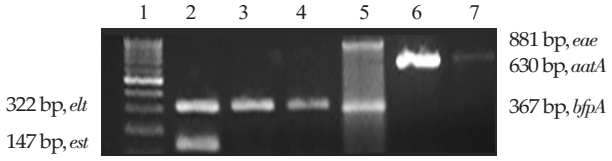

Figure 2. Multiplex PCR assay for detection of *Escherichia coli* in diarrhea sample. 1 = 100 bp DNA Ladder, 2 = IDH6 (positive for both *elt* and *est* gene), 3 = IDH2 (positive for *elt* gene), 4 = PDH40 (positive for *elt* gene), 5 = S14 (positive for both *bfpA* and *eae* gene), 6 = S5 (positive for *aatA* gene), 7 = IDH 21 (positive for *aatA* gene).

4. Discussion

Diarrhea and UTI remain as significant public health problems across the globe resulting in high morbidity and substantial mortality. It is well known that DEC and UPEC play a major role in diarrhea and UTI respectively. The present study shows that around one out of two patients admitted with diarrhea were infected with *E. coli* with the majority being due to 14.3% of ETEC (27/189) followed by 4.2% EPEC (8/189), 5.3% EAEC (10/189) and 0.5% EHEC(1/189). Similarly, one in eight UTI was due to *E. coli* including EHEC EAEC and ETEC strains.

The prevalence of DEC is only second to rota virus among children up to 5 years with diarrhea while DEC mediated diarrhea second only to cholera among children above 5 years[21]. Frequency of diarrhea due to DEC varies with geographical location, and population. Accordingly, the prevalence of DEC was described 40.0% and 34.0% of diarrhea in Bangladesh and Jordan respectively[20,22]. The present study reported a prevalence of DEC (24.4%) which is more than that reported in Kolkata, India[23], Mexico[24], and, China[25] but less than reports from Teheran[26], and Burkino Faso[27].

Among DEC, ETEC commonly causes diarrhea among children in developing countries followed by EAEC and EPEC. In our study, ETEC cases arising in diarrhea patients were found to be 27 (14.3%). Our study revealed a higher proportion of heat-stable toxin producing ETEC (6.3%) than reported in other countries[28,29], however, in urine samples only four cases of heat-stable toxin were

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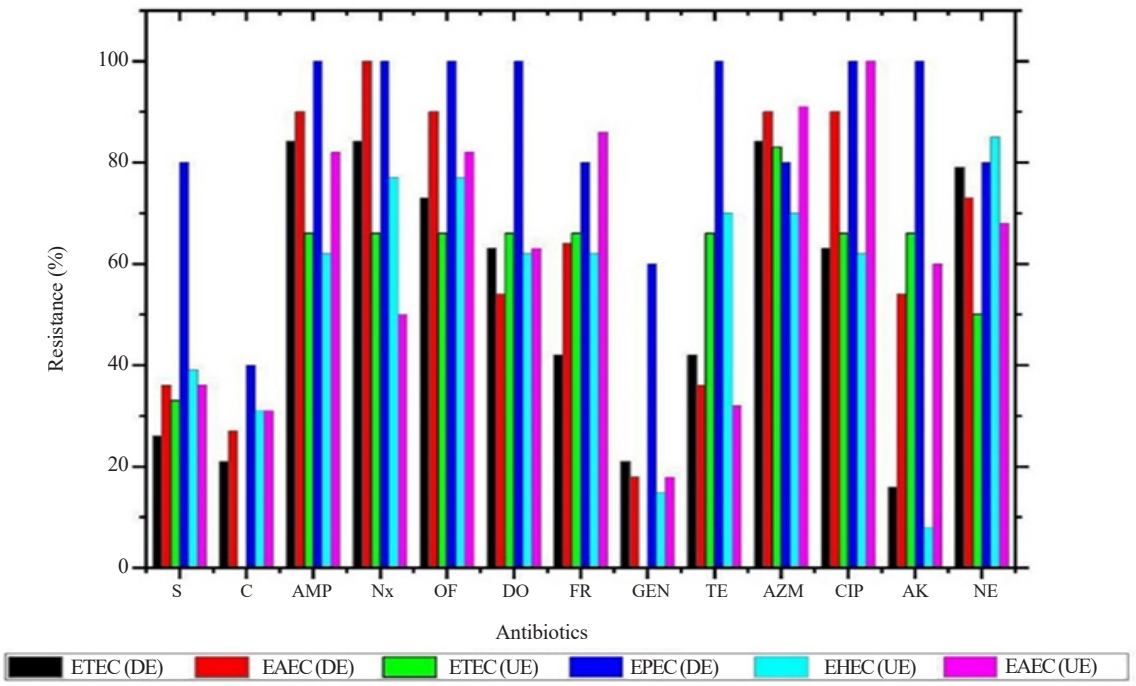

Figure 3. Antibiotic resistance pattern of different toxigenic strains of *E. coli* causing diarrhea and urinary tract infection. S: streptomycin; C: chloramphenicol; AMP: ampicillin; Nx: norfloxacin; OF: ofloxacin; DO: doxycycline; FR: furazolidone; GEM: gentamicin; TE: tetracycline; AZM: azithromycin; CIP: ciprofloxacin; AK: amikacin; NE: neomycin.

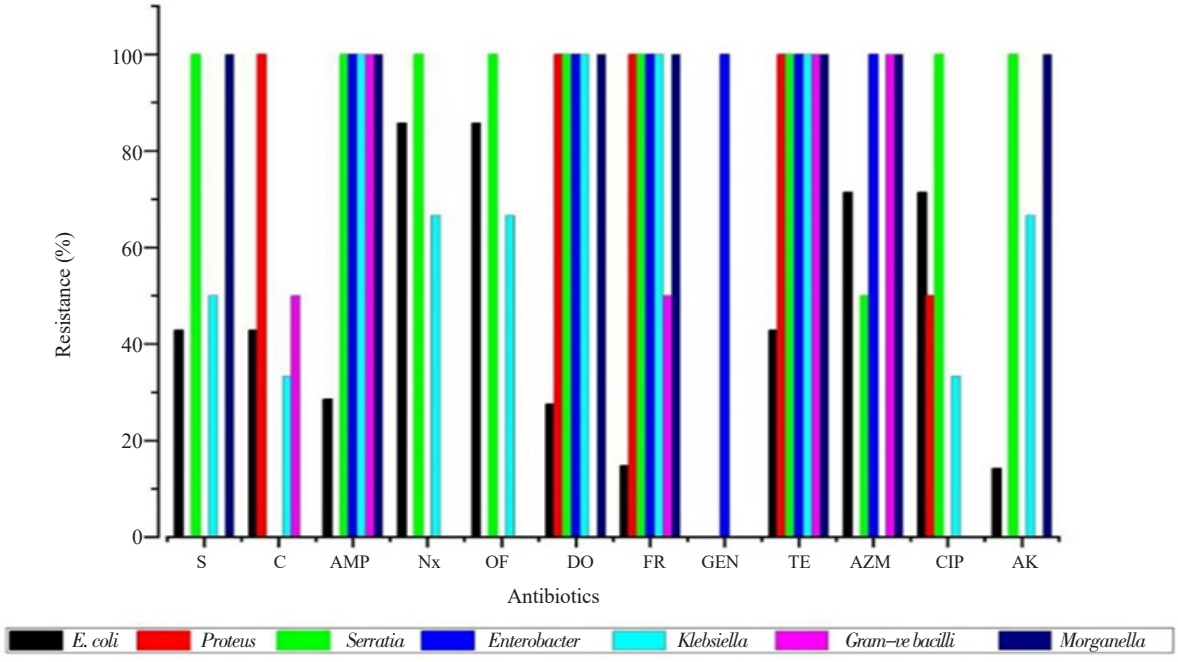

Figure 4. Antibiotic resistance pattern of different uropathogens causing urinary tract infection. S: streptomycin; C: chloramphenicol; AMP: ampicillin; Nx: norfloxacin; OF: ofloxacin; DO: doxycycline; FR: furazolidone; GEM: gentamicin; TE: tetracycline; AZM: azithromycin; CIP: ciprofloxacin; AK: amikacin.

seen. Our results were consistent with the results reported in Korea and Costa Rica and contrast to the reports of Iraq study that showed frequent detection of LT in ETEC associated with UTI[30]. Several studies have documented increasing levels of ST producing ETEC in children with acute diarrhea cases indicating its increasing public health importance[31].

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Besides being a diarrhea causing agent, EAEC strains have also been recorded as UTI causing bacterial pathogens in various parts of world[32]. Our study exhibited a frequency of EAEC (*aatA*) of 5.3% and 2.4% in diarrhea and UTI, respectively. In our study, EPEC was identified next to EAEC in stool samples while no EPEC was isolated in urine samples. In recent years several studies have shown that atypical EPEC (aEPEC) strains (eae^+) are appearing in greater proportion than typical EPEC (tEPEC) (eae^+ + $bfpA^+$) strains. In agreement with these reports, we reported here increased isolation of aEPEC strains in children with diarrhea.

Currently interest has been focussed on verotoxin producing *E. coli* O157:H7 and non-O157:H7 causing food-borne and waterborne illness. The prevalence of *E. coli* O157:H7 associated with diarrhea and UTI in different parts of the world is commonly found to be around 4%-7% and 2%-14% respectively[33–36]. In our study one case of *E. coli* O157:H7 was detected harbouring *eaeAO157*:H7. Interestingly, a greater number of EHEC 11 (3.7%) was isolated in patients with urine infection. These EHEC were comprised of 9 EHEC O157:H7 (*eaeAO157*+ and *vtx*−), 2 EHEC O157:H7 harbouring *eaeAO157* and *vtx2* and one non-O157:H7 possessing *vtx1* but did not possess *eaeAO157*. Disease caused by *E. coli* O157:H7 have much higher hospitalization and fatality rates[37], ranging from non-bloody diarrhea to life threatening HUS through bloody diarrhea. *E. coli* O157:H7 associated with HUS has been widely recognized as one of the main causes of acute renal failure[38]. In our study, the majority of the *E. coli* O157:H7 associated with urine infection were negative for *vtx*. The few *E. coli* O157:H7 that were positive for *vtx* did not give rise to HUS. Besides *E. coli* O157:H7, *E. coli* non-O157:H7 harbouring no *eaeAO157* also contribute for occurrence of disease due to presence of verotoxin.

The recent hike in drug resistance among DEC and UPEC has become the major concern that has challenged physicians to choose appropriate drug treatment. In several parts of world including South India[39], North India[40], Bolivia[41], and Iran[42]. DEC has been found resistant to multiple drugs such as norfloxacin, co-trimoxazole, ampicillin, ceftriaxone, metronidazole, gentamicin, ciprofloxacin, tetracycline, chloramphenicol, streptomycin in various proportions. In our study >70% isolates of DEC were resistant to ampicillin, azithromycin, norfloxacin, ciprofloxacin, ofloxacin, doxycycline and neomycin and while lower resistance <30% was encountered for chloramphenicol and gentamicin.

It is observed that UPEC strains are highly prone to develop antibiotic resistance. Several previous studies including investigations in Punjab, north India[43], Turkey[44], and, Mexico[45] revealed that UPECs are highly resistant to commonly prescribed antibiotics such as ampicillin nalidixic acid, cephalexin, and ciprofloxacin. In the present investigation we observed that UPEC strains were resistant to commonly used antibiotics like ampicillin, norfloxacin, ciprofloxacin, ofloxacin, azithromycin. Appearance of such type of multiple drug resistance in UPEC strains is a major threat.

5. Conclusions

To the best of our knowledge, this study is the first to report the incidence of DEC and UPEC and their antibiograms in Odisha, situated along the Bay of Bengal. DEC particularly higher report of ETEC (*est*+) and aEPEC in children of below five year is a matter of concern. Similarly, in UPEC, isolation of ETEC (*est*+) and EAEC (*aatA*+) associated with UTI is rarely reported in globe. The report of *E. coli* O157:H7 and non-O157:H7 in diarrhea and UTI is a matter of great concern. This suggests that constant surveillance for DEC and UPEC is necessary to adopt appropriate control measures. The current trend of antibiotic resistance in *E coli* isolates associated both in diarrhea and UTI is a public health concern and alternative medicines for treatment are urgently required.

Conflict of interest statement

The authors claim there is no conflict of interest.

Funding

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Biswas S and Khuntia HK contributed to conception and designing of the manuscript. Critical revision of the manuscript along with supplementing materials for preparing the manuscript thoroughly

was done by Ranjit M. All sorts of work related to sample collection from different hospitals and clinics of Odisha was done by Dixit S, Pattanaik SSS, Manna P, Routray S and Beuria J. The work of completing the manuscript was equally assisted by Pati S along with Bal M. All the authors agree with the content of the manuscript.

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