

Antimicrobial susceptibility patterns of *Escherichia coli* isolated from urine and stool samples of infected patient's in Lakhimpur district of Assam, India

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Abstract

To know the prevalence of antibiotic resistant *Escherichia coli* (E.coli) isolated from urine and stool samples of human beings in Lakhimpur district of Assam so that physician can easily go for best antibiotic advice as well as control measure can be taken in the specified infected area. Materials and Methods: Morning midstream urine samples and stool samples were collected in sterile containers and inoculated in different culture Medias and confirmed as E.Coli by Gram stain (Microscopic Observation), Growth characteristics of different medias, Biochemical tests like Methyl Red Test, Nitrate Reduction Tests, Indole Test etc. Sensitivity (drug sensitivity) was done in Muller Hinton Agar (MH Agar), where Fourteen (14) numbers different antibiotics used. Results: Results showed that E.coli is more sensitive to Amikacin in comparison with others antibiotics in both urine and stool samples. Conclusion: Amikacin can be used in Lakhimpur district of Assam for E.coli infection by the physicians and Bacitracin must be avoided.

Keywords: drug sensitivity, E.coli, Gram stain, Indole Test, MH Agar.

Introduction-

Escherichia coli (E.coli) is a Gram Negative (G-Ve) Bacilli, Sluggishly motile, normal microbial flora of the Intestinal Tract of warm-blooded organisms. The genus of this bacterium is *Escherichia*. Most of the E.Coli bacteria are harmless but some serotypes of them are responsible for UTI, food poisoning, pyogenic infection, sepsis and K etc [1]. In developing countries because of diarrhoea 2.2 million people die every year due to E.Coli like coliform infection [2]. Again every year 130–175 million patients suffer uncomplicated UTI worldwide and more than 80% them are due to E. coli [3]. India is on high alert against the deadly strain of Shiga toxin-producing E coli that has infected over 1,700 people across 12 European nations [4].

Humans and animals are the reservoir of E.coli, and it's typically associated with contaminated food and water. Prevention strategies of this pathogen include source protection, halogenations of water, or boiling

water for one minute for which awareness is an important factor. E. coli can affect anybody who expose to it but some people easily get its infections depending upon some factors like age, weakened immune system, eating habit etc. Generally children and older adults are more risky to get its infection. People having AIDS, Cancer etc. are also easily able to get ill after ingestion of E Coli with food. Another important factor of its infection is type of food which includes undercooked hamburger, unpasteurized milk, apple juice or cider, and soft cheeses made from raw milk etc [5].

The diseases/infections caused by a particular strain of E. coli depend on distribution and expression of an array of virulence determinants, including adhesions, invasins, toxins, and abilities to withstand host defenses. The common toxins of E Coli are LT toxin, ST toxin, Shiga toxin, cytotoxins, endotoxin (LPS) . Out of these toxin shiga toxin is an important toxin for causing diseases [6]. The selection of antibiotic is very important to control the infection. Our study tried to check the susceptibility pattern of E.coli in the specified area.

Materials and Methods-

Study Area: Lakhimpur district of Assam was selected for our study which is situated at North Eastern corner of Assam, The district covers 2977 Sq Km area which includes both hilly and plain. Because of which during rainy session or floods session waters comes down

from hilly to plain which is a common cause of contamination. Lack of awareness or knowledge is the main cause of the taking contaminated water, food, living in unhygienic condition etc are seen here because of which we have selected this area as our study area.

Urine Collection: Patients reported with Urinary tract infection at North Lakhimpur civil Hospital were advised to send their morning midstream urine sample for culture and sensitivity test to District Priority Laboratory of North Lakhimpur Civil Hospital. Sterile containers were provided to the patients and advised by laboratory personnel to clean their genital organ with soap before collect the midstream sample in the container. Inoculation was done in Nutrient Agar, UTI Agar and MacConkey's Agar within 1-2 hours after collection of the sample or as early as reported to the laboratory.

Stool Collection: Patients reported to the hospital with diarrhoea were advised to send their stool samples to the Laboratory for culture and sensitivity test in sterile container. Laboratory personnel were instruct them to collect the sample in the container without any contamination with urine, water or any others. Blood Agar Media were used for stool culture (inoculation) in our study.

Gram Stain and Microscopic Observation: Smears were prepared in clean sterile Microscopic glass slide by spreading the loopful growth over a small area of the slide with a drop of water, and allowed to dry. The dry smears were fixed with the help of flame. Flooded the slide with crystal violet solution for up to one minute and then washed off with tape water (aprox. 5 seconds). Secondly Gram's Iodine solution was added washed off with tap water and Drained. In the third step excess water were removed from slide and

then blotted. Slides were flooded with 95% alcohol for 10 seconds and wash off with tap water. Drained the slide. Safranin was added in the fourth step for counterstain for 30 seconds. Drained the slide and blotted to dry with bibulous paper. After drying Microscopic observation was done under oil immersion where Gram Negative Bacilli were rod shaped in red color (Safranin colour).

Methyl Red Test: An inoculum from a pure culture was transferred aseptically to a sterile tube containing MRVP broth. The inoculated tubes were incubated at 37°C for 24 hours. At the end of the incubation, five drops of methyl red were added. Mixed acids fermentation results in a red color change indicated positive Methyl Red Test (Example: E.Coli).

Nitrate Reduction Test: Nitrate Broths were used run the test, where inoculation was done from the bacterial growth of the agar plate. And incubated for 24 hours at 37°C. After incubation one drop of sulfanilic acid and one drop of α-naphthylamine was added to the broth. where red colour indicated positive nitrate reduction test, if there were no colour change then small amount of zinc were added colour change to red at this point indicated negative but no colour change at this point indicated positive result.

Indole Test: An inoculum from a pure culture was transferred aseptically to a sterile tube containing peptone broth and incubated at 37°C for 24 hours. At the end of the incubation five drops of Kovac's reagents were added and presence of a red or red-violet color in the surface alcohol layer of the broth indicated indole positive (Example: E.Coli).

Sensitivity: All the sample's isolates were tested for Antibiotic Sensitivity Test by Kirby and Buyer Method in Muller Hinton Agar Media (Hi-Media).

Tables-

Sr. No.	Antibiotic	Sensitive %	Resistant %
1	Amikacin (AK30)	73	27
2	Penicillin (P10)	19	81
3	Ampicillin (AMP25)	23	77
4	Cefixime (CFM5)	23	77
5	Amoxyclav (AMC30)	35	65
6	Ciprofloxacin (CIP5)	54	46
7	Norfloxacin (NX10)	19	81
8	Ceftriaxone (CTR30)	38	62
9	Cefotaxime (CTX30)	42	58
10	Tetracycline (TE30)	3	97
11	Gentamicin (GEN10)	31	69
12	Co-Trimoxazole (COT25)	3	97
13	Chloramphenicol (C30)	19	81
14	Bacitracin (B10)	3	97

Table 1 – Antibiotics sensitivity of E. Coli Seen in urine samples

Sr. No.	Antibiotic	Sensitive %	Resistant %
1	Amikacin (AK30)	92	8
2	Penicillin (P10)	7	93
3	Ampicillin (AMP25)	8	92
4	Cefixime (CFM5)	31	69
5	Amoxyclav (AMC30)	31	69
6	Ciprofloxacin (CIP5)	69	31
7	Norfloxacin (NX10)	23	77
8	Ceftriaxone (CTR30)	15	85
9	Cefotaxime (CTX30)	38	62
10	Tetracycline (TE30)	38	62
11	Gentamicin (GEN10)	38	62
12	Co-Trimoxazole (COT25)	8	92
13	Chloramphenicol (C30)	16	84
14	Bacitracin (B10)	7	93

Table 2 – Antibiotics sensitivity of E. Coli Seen in stool samples

Images-



Image1- Sensitivity in MH Agar Media

Figures-

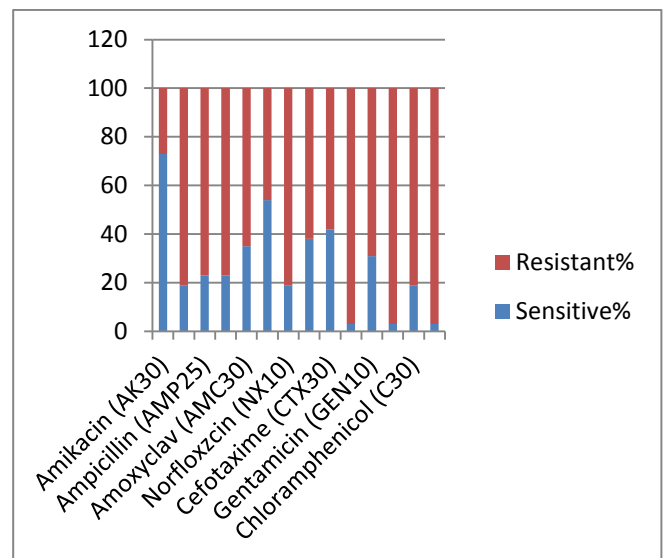


Figure 1- Sensitivity and Resistant data of E.Coli in Urine Samples

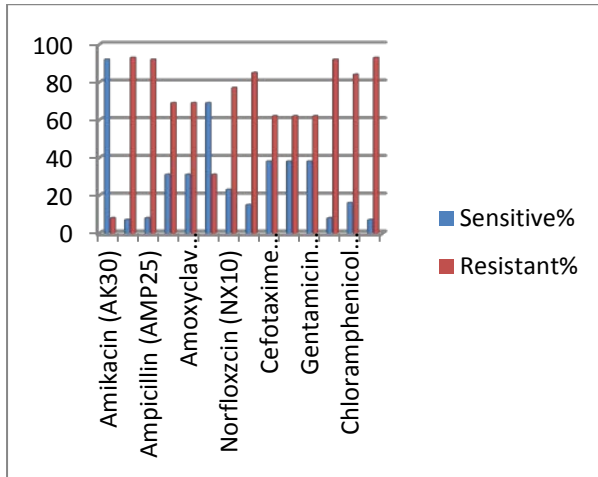


Figure 2- Sensitivity and Resistant data of E.Coli in Stool Samples

Results-

E.Coli was confirmed by growth characteristics nature in different agar media used to inoculation, Microscopic observation after gram stain and different biochemical test positivity. Methyl Red Test, Nitrate Reduction Tests, Indole Test and oxidase test were done out of which except oxidase test E.Coli showed positive results in all tests. Antibiotics Sensitivity were checked in Muller Hinton Agar Media. Fourteen numbers of antibiotics were used to check the results, The antibiotics were Amikacin, Penicillin, Ampicillin (AMP25), Cefixime (CFM5), Amoxyclav (AMC30), Ciprofloxacin (CIP5), Norfloxacin (NX10), Ceftriaxone (CTR30), Cefotaxime (CTX30), Tetracycline (TE30), Gentamicin (GEN10), Co-Trimoxazole (COT25), Chloramphenicol (C30) and Bacitracin (B10). Out of all fourteen antibiotics in urine sample’s E.coli most sensitive antibiotics were Amikacin (AK30) followed by Ciprofloxacin (CIP5) and Cefotaxime (CTX30), same way in stool sample’s E.Coli most sensitive antibiotics were Amikacin (AK30) followed by Ciprofloxacin (CIP5) and then others.

Discussion-

The study done by S Sharma et al., 2007 showed that 76.9% patients E.coli isolates were resistant to Ampicillin where as Cefotaxime were most sensitive to the bacteria [7]. But in our study it was found that Bacitracin was most resistant antibiotic. The drug resistant pattern based geographical area may varies due to different factors like over dose of specified drug, environmental factors etc. The study of S Sharma was done at Mangalore where as our study at Lakhimpur, Assam. From Assam (Guwahati) in the study of BORA A et al., 2012 found 74.3% E.Coli sensitive to Amikacin and 100% sensitive to Imipenem [3]. But our study showed 73% sensitive with E.Coli isolated from urine and 92% sensitive with E.Coli isolated from stool samples. The average will be 82.5 % . Though Guwahati and Lakhimpur are not neighbouring district the difference of 8.2%. Another study of NB Hirulkar from indore found more then 70% sensitivity towards Piperacillin, Tetracycline, Vancomycim and Chloremphenicol in our study it was 20.5% for tetracycline and 17.5% for Chloremphenicol [2].

Conclusion-

The drug resistances displayed by the bacteria, Escherichia coli are indicated indiscriminate use of these antibiotics, it may due to patients self treating behaviour from pharmacy without any knowledge of dose or sensitive resistant knowledge. Which Warrants the initiation of steps to prevent public health hazard (Tambekar and Charan in 2004 And Pandey and Mussarat in 1993). Time to time the sensitive resistant nature of bacteria may change due to non use or over use of drugs for which time to time study is necessary.

References-

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