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A Study on Isolation of E. Coli Bacteria from Different Human Clinical Specimens

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ABSTRACT

E.coli is a gram negative bacillus found in the human and animal gut and is the leading cause of Urinary tract infections and various other infections and complications. This is a retrospective study comprising of 1040 human clinical specimens of urine, pus, blood and sputum received over a period of six months in our hospital which came for culture and sensitivity. Out of these specimens 5.09% total specimens, 8.9% urine specimens, 5.2% pus specimens, 0.8% blood specimens and 1.5% sputum specimens were positive for E. coli growth. We also checked for drug sensitivity of various drugs and found out that Aminoglycosides, Carbapenems, Fosfomycin, Colistin and Nitrofurantoin showed good sensitivity against E. coli. From our study we concluded that E. coli is a common cause for urinary tract infections and wise usage of antibiotics should be done to prevent the growing problem of antibiotic resistance.

KEY WORDS: Escherichia coli, urinary tract infection, culture, antibiotic sensitivity.

INTRODUCTION

In this study, held in the Microbiology Department of DNS hospitals Pvt. Limited, Indore, we have tried to study the isolation of E.coli which is a gram negative bacillus in different human clinical specimens. E.coli genus has been named after Escherichia who was the first one to describe the colon bacillus under this name in the year 1885. E.coli is further sub divided into biotypes and serotypes. E.coli is gram negative straight, rod shaped, arranged singly or in pairs, it is motile by peritrichate flagella. It is an aerobe and facultative anaerobe which grows at 37°C temperature on culture mediums like Nutrient Agar, MacConkey Agar, and Blood Agar. [(1)]

Taxonomic classification of bacteria[(1)]

Domain	Bacteria
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gamma proteobacteria
Order	Enterobacteriales
Phylum	Enterobacriaceae
Genus	Escherichia
Species	Escherichia coli

Escherichia coli is one of the main causes of nosocomial infections in humans. E. coli is also a common inhabitant of the human and animal gut and is considered an indicator of fecal contamination in food. [(2)] E.coli is a parasite living only in the human or animal intestine. It is a very common pathogen established as a causative agent for urinary tract infections and is among the most common pathogens causing blood stream infections, wounds, otitis media and other complications in humans. [(3,4,5)] Antimicrobial resistance is an evolving and growing problem in UTI, of more concern is

the increasing incidence of infections attributed to the strains of E. coli that are commonly resistant to beta- lactams and Cotrimoxazole .[6,7]

MATERIALS AND METHODS: [8,9]

In this retrospective study, we have taken a data of 1040 human clinical specimens that were received in the Microbiology Department at DNS hospitals pvt. ltd., Indore over a period of 6 months that is from October 2020 to March 2021. These specimens included urine samples, blood samples, pus samples and sputum samples.

Urine samples were taken by voiding the first part of urine stream and then collecting the mid stream sample in a sterile container.

For Pus samples the area around the site of collection was cleaned by 5% Betadine solution from less contaminated to more contaminated area and then the sample was collected by a sterile syringe, or sterile cotton swab.

For blood samples the ideal procedure followed was cleaning the site with 5% Betadine solution followed by cleaning with a dry sterile swab and again with 5% Betadine solution and then blood was withdrawn from the particular site.

For sputum collection, the muco salivary sample was avoided and thick mucoid sample was collected in a sterile container.

Then after receiving these samples in a sterile manner in the microbiology lab they were further processed in the following manner –

The urine specimens were spread by the spread plate method on MacConkey agar and hi-chrome agar. The spread plate method is a technique to plate a liquid sample containing bacteria so that the bacteria are easy to count and isolate. A successful spread plate will have a countable number of isolated bacterial colonies, evenly distributed on the plate. The other specimens like pus and sputum were streaked on nutrient agar, blood agar and MacConkey agar by streak plate method. Streak plate technique is used to grow bacteria on a growth media surface so that individual bacterial colonies are isolated and sampled. Samples can then be taken from the resulting isolated colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested. In case of blood cultures, the samples were taken in blood culture bottles and then loaded into BD Phoenix FX 40 automated blood culture analyser which gives positive or negative result based on fluorescent technologies within 5 days and in case of growth similar method of streaking is done on different agars.

These plates are incubated at 37 degree C for 18-24 hours in aerobic condition and then checked for bacterial growth. In case of bacterial growth, further biochemical tests like catalase, coagulase, and oxidase were done along with lactose fermentation test for further classification. Also the colony count was checked for positive urine specimens.

After the growth, the pure colonies were picked by a sterile loop to make a suspension with normal saline on a glass slide and were followed by gram staining to check for gram positive and gram negative bacterial identification.

Next the pure colony was picked up by a sterile loop and mixed with the ID broth to make a suspension of density between 0.5 to 0.6 which was measured by a nephelometer and 25ul was transferred to both of the AST Broths to which one drop of AST indicator is added and then after gently mixing 3.5ml of one of the AST broth was discarded and the remaining was added to the channel one of NMIC 500 panel and 8 ml of the second AST broth was added to the channel two o NMIC 500 panel. Then the remaining suspension was transferred into NID panel for species identification. Then these two ID and AST panels were loaded in BD phoenix M 50 analyser which does the identification in 4-5 hours and gave the antibiotic susceptibility in 18-20 hours.

Figure 1

Figure 2

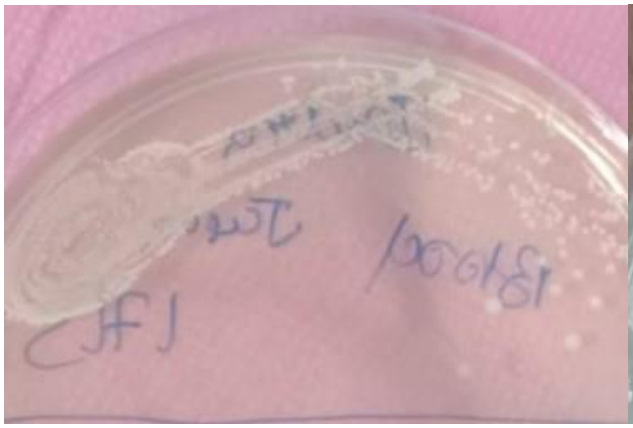


Figure 3

Figure 4

Figure 5

Figure 1: showing E.coli growth on MacConkey agar by spread plate method in urine specimen.
Figure 2: showing E.coli growth on MacConkey by streak plate method specimen in pus specimen.
Figure 3: showing E.coli growth on Nutrient agar by streak plate method in pus specimen.
Figure 4: showing E.coli growth on Nutrient agar by streak plate method in blood specimen.
Figure 5: Picture of automated BD phoenix M 50 ID /AST analyser.

RESULT

Out of a total number of 1040 human clinical specimens that we received over a period of six months in the Microbiology Department at DNS hospitals, Indore the maximum were specimens of urine that is 447 (42.98%) followed by specimens for blood culture i.e. 360 (34.61%). While pus samples contributed 16.34% i.e. total 170 specimens and the least being 63 sputum samples i.e. 6.05%.

Out of the total specimens received, positivity was seen in total 53 specimens (5.09%). Further among these four types of

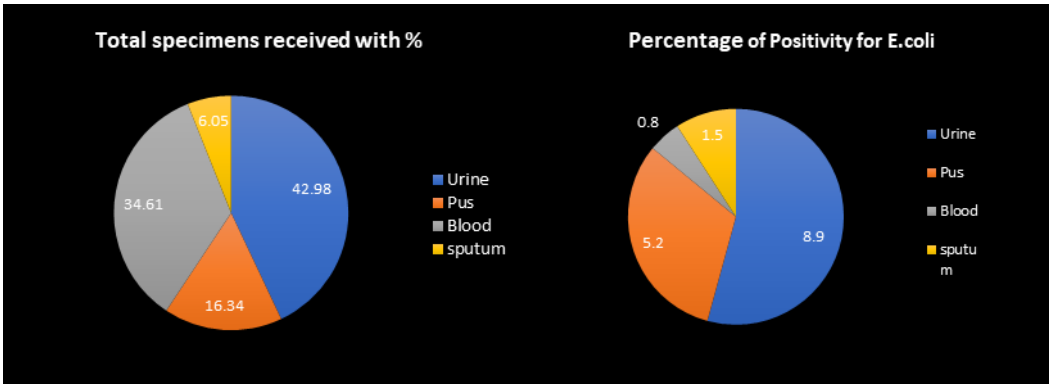
human clinical specimens the maximum percentage of E.coli growth was observed in urine specimens with 40(8.9%) samples being positive, followed by pus specimens with total 9(5.2%) specimens being positive. sputum and blood samples showed very less percentage positivity for E.coli growth i.e. 1.5 % and 0.8 % respectively.

Also we took into account the sensitivity and resistance pattern shown by various drugs. Considering the positive urine samples, maximum amount of sensitivity was seen in drugs like Fosfomycin and Colistin with 87.5% (35 out of 40) of positive urine samples being sensitive, closely followed be Aminoglycosides with Amikacin and Gentamycin showing sensitivity in 82.5% (33), and 77.5% (31) of positive samples. The Carbapenems group of drugs like Ertapenem, Imipenem and Meropenem demonstrated sensitivity in fair amount of positive samples i.e. 70 %(28), 65 %(26) and 75 %(30) respectively. Special drug Nitrofurantoin used for UTI showed good sensitivity i.e. in 80% (32) positive specimens.

Then taking into account specimens of pus, 9 were positive for E.coli. , in these positive samples Colistin was sensitive in 8 specimens (88.8%). Aminoglycosides like Amikacin and gentamycin were sensitive in 7 (77.7%) and 5 (55.5%) positive specimens. Also the Carbapenems class of drugs showed sensitivity with Ertapenem being sensitive in 5 (55.5%), Meropenem in 6 (66.6%) and Imipenem in 7 (77.7%) of the positive specimens.

Table 1:- Table showing numbers and percentage of specimens received along with numbers and percentage of positivity for E.coli in these samples.

Types of specimens	Total no. of specimens received with %	Total no. and % of Positivity for E.coli
Urine	447(42.98%)	40(8.9%)
Pus	170(16.34%)	9(5.2%)
Blood	360(34.61%)	3(0.8%)
Sputum	63(6.05)	1(1.5%)
Total	1040	53 (5.09%)



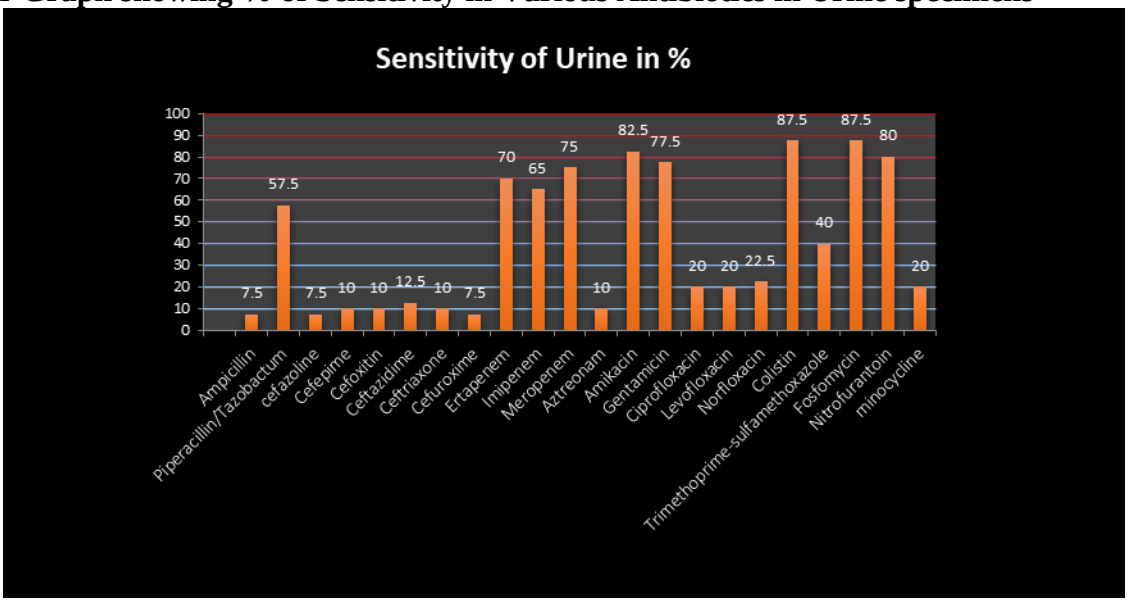
(1) **(2)**

Table 2:- Table showing numbers and percentage of sensitivity in various antibiotics in Urine and Pus specimens.

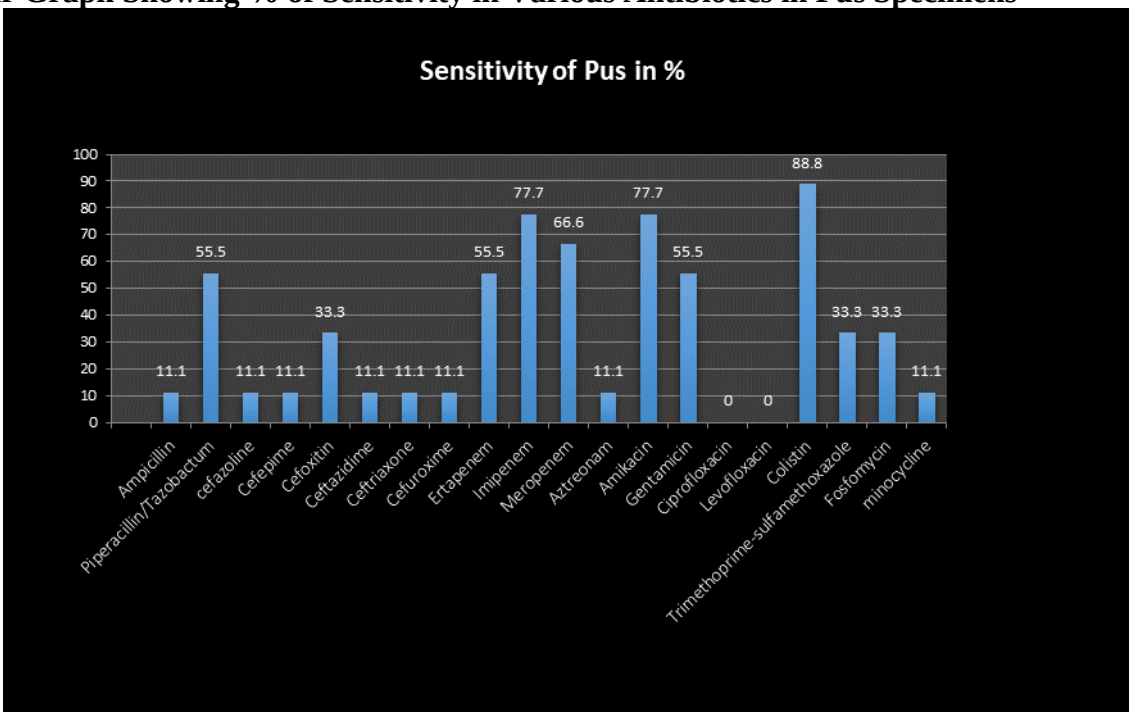
Antibiotics	Sensitivity in Urine	Sensitivity in Pus
Ampicillin	3(7.5%)	1(11.1%)
Piperacillin/Tazobactam	23(57.5%)	5(55.5%)
Cefazolin	3(7.5%)	1(11.1%)
Cefepime	4 (10%)	1(11.1%)
Cefoxitin	4(10%)	3(33.3%)
Ceftazidime	5(12.5%)	1(11.1%)
Ceftriaxone	4(10%)	1(11.1%)
Cefuroxime	3(7.5%)	1(11.1%)
Ertapenem	28(70%)	5(55.5%)
Imipenem	26(65%)	7(77.7%)
Meropenem	30(75%)	6(66.7%)
Aztreonam	4(10%)	1(11.1%)
Amikacin	33(82.5%)	7(77.7%)
Gentamicin	31(77.5%)	5(55.5%)

Table 2. Continued…		
Ciprofloxacin	8(20%)	0(0.0%)
Levofloxacin	8(20%)	0(0.0%)
Norfloxacin	9(22.5%)	NA
Colistin	35(87.5%)	8(88.8%)
Trimethoprim-sulfamethoxazole	16(40%)	3(33.3%)
Fosfomycin	35(87.5%)	3(33.3%)
Nitrofurantoin	32(80%)	NA
Minocycline	8(20%)	1(11.1%)

Bar Graph showing % of Sensitivity in Various Antibiotics in Urine specimens



Bar Graph Showing % of Sensitivity in Various Antibiotics in Pus Specimens



DISCUSSION

Culture characteristics: It is an aerobe and facultative anaerobe which grow in 10-40 degree C on ordinary media the colonies are thick, large greyish white, moist smooth opaque or partially translucent. There are two forms the smooth form seen on fresh isolation and emulsifiable in saline and the

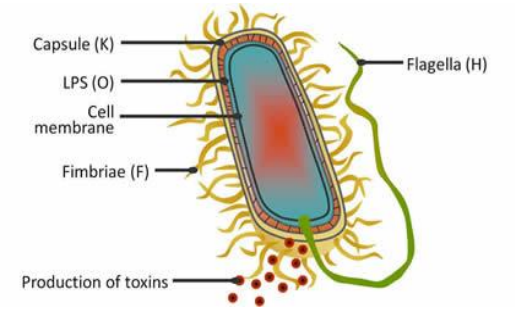
rough forms which are autoagglutinable in saline. This is due to lot of surface antigen. On MacConkey medium colonies are light pink due to lactose fermentation while in broth growth occurs as turbidity and heavy deposit which disperses on shaking. Many strains are hemolytic on blood agar.

Biochemical reactions [(10)]

Characteristics	***E. coli***
Gram Staining	Negative
Shape (Cocci/Diplococci/Rods)	Rods
Motility (Motile / Non-Motile)	Motile
Capsule (Capsulated/Non-Capsulated)	Variable
Spore (Sporing/Non-Sporing)	Non-Sporing
Flagella (Flagellated/Non-Flagellated)	Flagellated
Catalase	Positive (+ve)
Oxidase	Negative (-ve)
MR (methyl red)	Positive (+ve)
VP (vogus proskur's)	Negative (-ve)
OF (Oxidative/Fermentative)	Fermentative
Indole	Positive (+ve)
Simmon Citrate	Negative (-ve)
Urease	Negative (-ve)
Nitrate Reduction	Positive (+ve)
H2S	Negative (-ve)
Gas	Positive (+ve)
Coagulase	Negative (-ve)
Hemolysis (Alfa/Beta/Gamma)	Some Strains shows Hemolysis
Fermentation of	
Glucose	Positive (+ve)
Lactose	Positive (+ve)
Mannitol	Positive (+ve)
Sorbitol	Positive (+ve)
Sucrose	Variable

Antigenic structure[(10)]

Serotyping of E.coli is based on three antigens the somatic antigen O, capsular antigen K and flagger antigen H.



Virulence factor[(10)]

Surface antigen toxins O have endotoxic activity, K protects against phagocytosis, fimbriae antigen H promotes virulence.

Toxins of E.coli produce exotoxins, hemolysins and enterotoxins. These are produced by ETEC strains which lead to watery diarrhea. There are two types of enterotoxins heat labile and heat stable, these are composed of five beta units for binding and one alpha sub unite for the enzymatic activity. The labile toxin causes increased outflow of water and electrolytes in the gut lumen and causes diarrhea while the stable toxin causes fluid accumulation in intestine. Some E.coli also produce verocytotoxin which causes cytotoxicity to Vero cells hence act like shigella dysentery toxin.

Classification of E.coli [(11, 12, 13,14,15,16,17,18,19, 20)]

Enteropathogenic EPEC It primarily affects infants and young children in resource-limited settings and causes sporadic and epidemic outbreaks. It was the first pathotype identified as a causative agent of watery diarrhea.

Entrotoxigenic ETEC It is commonly found in food and water in areas without adequate sanitation it leads watery diarrhea in resource-limited conditions.

Enteroinvasive EIEC - It rarely causes diarrhea due to the relatively large inoculums required, although it may be under diagnosed.

Enterohemorragic EHEC- It is responsible for large diarrheal outbreaks after ingesting contaminated food like spinach, sprouts, lettuce, fruits, raw dairy products and undercooked beef. Relatively low inoculums (102 CFU) of EHEC/STEC result in illness, hence causing transmission from the environment to humans and humans to humans. Hemolytic uremic syndrome (HUS) caused by EHEC/STEC infections is most common in children less than five years old and adults greater than 60 years old.

Enteroaggresive EAEC - It is causative organism of acute and chronic watery diarrhea and has been increasingly identified as a cause of traveler's diarrhea.

E. coli has widely been implicated in various clinical infections as hospital acquired and community infections as reported by Shah et al. (2002). Pathogenic isolates of E. coli have relatively high potentials for developing resistance (Karlowsky et al., 2004). [2,21,22] Antimicrobial resistance in E. coli has increased worldwide and its susceptibility patterns show substantial geographic variation as well as differences in population and environment [3, 23] The low growth might be due to inclusion of every patients requesting for culture regardless of their symptoms and illness or prior use of antibiotics or might be due to presence of fastidious organisms that we are not be able to grow on routine culture media.[6,24] The urinary tract constitutes the most common site of human bacterial infection, and Escherichia coli is, by far, the most prevalent causative organism at this site. Most urinary tract infections result from ascension of bacteria from the urethra to the bladder and possibly kidneys. [25, 26, 27, 28] Similarly in our study the maximum number of growths was seen in urine specimens. A number of studies suggested that sexual activity is one of the important influential factors for UTI in women. [7, 29, 30, 31]

CONCLUSION

Hence we conclude from this study that maximum percentage of growth for E.coli was observed in urine specimen and out of the total specimen positive for E.coli, we also check for sensitivity pattern for E.coli in various drugs and we concluded that group of antibiotic such as carbapenems, aminoglycosides and miscellaneous drugs like Colistin, fosfomycin and Nitrofurantoin these were showed most sensitivity in maximum no. of cases in E.coli. These were most sensitivity in urine while in Pus specimen carbapenems, aminoglycosides And miscellaneous drugs like Colistin showed maximum no. of sensitivity.

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Conflict of Interest: None

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Ethical Approval: Approved

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