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ANTIBIOTIC RESISTANT WOUND ASSOCIATED ESCHERICHIA COLI ISOLATED FROM PUS AND ITS ANTIBIOGRAM

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ABSTRACT

Escherichia coli is human pathogenic microorganism causing urinary tract infection, wound infection, pneumonia, meningitis and septicemia. Some pathogenic strain causes gastrointestinal diseases. The incidence of gastroenteritis varies in different graphical area. It also causes diarrhea by complex mechanism that may involve production of varieties of enterotoxin. An outpouring of fluid and electrolytes occur from wall of small intestine. Toxin production by E. coli depends on presence of drug resistant plasmid. E. coli from most common cause of intra-abdominal infection such as peritonitis and abscess resulting from spillage of bowel content they also cause pyogenic infection in perianal area. In present study isolation of E. coli was carried out from pus using blood agar, MacConkeys agar, and Nutrient agar during Aug. 2015. And its sensitivity was tested with twenty two different antimicrobial agents. The isolated strain shows sensitivity against only four antibiotic viz. Imipenem, Meropenem, Ertapenem and Amikacin, while it showed resistance against Ampicillin, Amoxicillin, Piperacillin, Cefixime, Ofloxacin, Monocyline etc.

KEY WORDS: Escherichia coli, enterotoxin, drug resistant plasmid.

INTRODUCTION

Escherichia coli belongs from family enterobacteriaceae. This organism is firstly discovered by Theoder Escherich in 1885 from faeces of newborn three days old child. Genus Escherichia includes six species, of which five are associated with human diseases E. coli, E. decarboxylata, E. hermanii, E. fergusonii, and E. blallate (Day et al, 1999). E. coli is gram negative short straight road, measuring 0.4 -0.7 x 1-3 micrometer, found singly or in pair, motile with peritrichous flagella, capsule and fimbriae may be found in some strain but no spore. Escherichia coli is human pathogenic microorganism causing urinary tract infection (UTI) wound infection, pneumonia, meningitis and septicemia. Some pathogenic strain causes gastrointestinal diseases. The incidence of gastroenteritis varies in different graphical area. It also causes diarrhea by complex mechanism that may involve production of varieties of enterotoxin. An outporing of fluid and electrolytes occur from wall of small intestine. Toxin production by E. coli depends on presence of drug resistant plasmid. E. coli from most common cause of intra-abdominal infection such as peritonitis and abscess resulting from spillage of bowel content they also cause pyogenic infection in perianal area.

Diarrhea-associated E. coli is confusing and complex, in part because it is evolving. The organisms are defined by the presence and expression of specific virulence genes. Treatment issues can be understood only in the context of the clinical, epidemiologic, and laboratory data that help in diagnosis. Understanding these issues is necessary to address the treatment issues, including antimicrobial resistance. There are five categories of diarrhea-associated E. coli i.e. enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enterohemorrhagic E. coil (EHEC), and enteroaggregative E. coli (EAggEC) (Henry and Thomas, 1996).

Illness has been characterized by irritability, anorexia (Nelson and Haltalin, 1971) with minimal emesis or fever, and stools that are watery, yellow-green, and without mucous, pus, or blood, (Guerrant, 1990). Life threatening dehydration and shock can complicate the course. In malnourished infants, electrolyte disturbances and very prolonged diarrhea commonly follow acute illness (Khoshoo and Bhan, 1990). Disease caused by ETEC resembles mild cholera, with watery diarrhea, abdominal pain, nausea, and vomiting (Guerrent, 1990). Illness caused by EIEC dysentery that resembles shigellosis. Thus, food-borne transmission rather than person-to-person transmission is seen with EIEC (Henry and Thomas, 1996). Dysentery due to EIEC is uncommon in the United States but more common in Central and South America and Asia (Saelzer et al, 1989; Echeverria et al, 1991; Kain et al, 1991). EHEC cause bloody diarrhea without fever may be preceded by watery diarrhea and abdominal pain (Cohen and Giannella, 1992). Like EPEC, the EAggEC have distinctive adherence patterns in tissue culture systems (Mathewson et al, 1985). Approximately 10-25% of urinary tract isolates of Escherichia coli from female outpatients are resistant to trimethoprimsulfamethoxazole (SXT) (Diekema et al, 2004; Hooton, 2003). Increasing rates of resistance to ampicillin, cefdinir, and nitrofurantoin were observed against E. coli isolated from urine specimen (James et al, 2006). Antibiotic susceptibility profile of uropathogenic Proteus mirabilis showed least effective against ampicillin, cefuroxime, ofloxacin and chloramphenicol (Singla et al, 2015). Urinary infection result when bacterial virulence factor overcomes the numerous host defense mechanism (Ugoh et al, 2013).

MATERIALS AND METHODS

COLLECTION OF SAMPLE

Pus sample was collected using standard microbiological techniques from 65 year old female patient from Jalna district of Maharashtra during last week of August 2015. Sample was collected using sterile cotton swab and aspirates using sterile syringe. The sample was brought to microbiology laboratory within one hour.

ISOLATION OF *E. coli*

Isolation of E. coli was carried out by using bacteriological media like Blood agar (BA), MacConkeys agar (MA), and Nutrient agar (NA). The pus sample collected in swab and sterile disposable syringe was inoculated into these media and plates of BA, MA and NA was incubated at 370C for 18 hours (Cheeseborough 2002).

IDENTIFICATION OF MICROORGANISM

Isolated colonies developed on BA, MA, and NA medium was picked up and characterized by cultural characterization and biochemical characterization following standard methods (Edward and Ewing, 1972). The biochemical tests carried out for identification was IMViC test, fermentation of carbohydrates (glucose, lactose, sucrose, mannitol and maltose),

H2S production. Enzyme production like urease. These tests were carried out following standard routine techniques.

ANTIBIOGRAM OF *E. coli*

Susceptibility testing of isolated species was carried out using disk diffusion method. Twenty two different antibiotics were used to test susceptibility was, Ampicillin, Ampicillin / Salbactum, Amoxicillin / Clavulanic acid, Piperacillin / Tazobactam, Cephalothin 1st generation, Cefuroxime 2nd generation, Cefoperazone 3rd generation, Cefotaxime 3rd generation, Cefixime 3rd generation, Cefepime 4th generation, Imipenem, Meropenem, Ertapenem, Gentamycin, Amikacin, Ofloxacin 2nd generation, Ciprofloxacin 2nd generation, Levofloxacin 3rd generation, Co-Trimoxazole, Cefoperazone/ Salbactum, Doxycycline, and Minocycline. The susceptibility and resistancy of *E. coli* was determined on the basis of zone of inhibition around the disc.

RESULT AND DISCUSSION

After inoculation of pus sample in plates, plates were incubated for 18 hours at 37^0C shows development of colonies on all media. Cultural and biochemical characterization of colonies developed on BA, MA and NA plates was noted.

ISOLATION AND IDENTIFICATION OF MICRO ORGANISM

Isolation of *E. coli* was carried out by using different media like MacConkeys agar (MA), Blood agar (BA) and Nutrient agar (NA). The pus sample collected in swab and aspirated by using sterile disposable syringe was inoculated in to MA, BA and NA plates, and incubated at 37^0C for overnight (Cheesebrough 2006).

Identification of *E. coli* was carried by cultural and biochemical characterization. On MA plates, colonies developed were pink due to fermentation of disaccharide lactose, measures 1-2 mm in size, circular, and convex with entire margin. On BA hemolysis was observed this indicates the isolated strain was pathogenic in nature. On NA, colonies were large thick, grayish, white, moist, smooth, opaque few were partially translucent.

Biochemically isolated strain showed fermentation of glucose, lactose, mannitol, maltose and no fermentation of sucrose. The widely used four biochemical test were IMViC. This test is commonly used to differentiate and classify *E. coli* from *Aerobacter aerogen*. The isolated strain showed Indole production and positive with methyl red (MR) test, while it was negative with Voges-Proskauer (VP) and citrate utilization. Negative result was also reported with H_2S and urease production.

ANTIBIOGRAM

After isolation, and identification of *E. coli* by cultural and biochemical characterization, an isolate was inoculated in to Mueller-Hinton agar M-173 (MHA) by Bauer-Kirbys disc diffusion method according to clinical laboratory standard institute (CLSI) guidelines(CLSI, 2006) to check the sensitivity of microorganism. Twenty two different antibiotics were used to test the susceptibility and resistance of *E. coli*. The antimicrobial disc (Hi-Media) with their concentration was used to check sensitivity of microorganism to antibiotics. Sensitive microorganism shows zone of inhibition around the disc. The zone size interpretative chart of antibiotics (Hi-Media) is shown in table number 1. And their effect on *E. coli* is depicted in table number 2.

Table No. 1. Zone size interpretative chart of few antibiotics (Hi-Media).

Name of antibiotics	Disc content	Resistant, mm or less	Sensitive, mm or less

Amikacin	30 mcg	14	17
Amoxicillin	10 mcg	13	18
Ampicillin	10 mcg	13	17
Ampicillin / Salbactum	10 mcg	11	15
Cefipime	30 mcg	14	18
Cefixime	5 mcg	15	19
Cefoperazone	75 mcg	15	21
Ceftriaxone	30 mcg	13	21
Ciprofloxacin	5 mcg	15	21
Co-Trimoxazole	1.25 mcg	10	16
Fospomycin	200 mcg	12	16
Gentamycin	10 mcg	12	15
Kanamycin	30 mcg	13	18
Levofloxacin	5 mcg	13	17
Norfloxacin	10 mcg	12	17
Oflaxacin	5 mcg	12	16
Piperacillin	100 mcg	17	18
Piper / Tazobactam	100/10 mcg	17	21
Rifampicin	5 mcg	16	19
Streptomycin	100 mcg	14	18
Amoxicillin / Clavulanic acid	20/3 mcg	19	20
Cephalothin 1st Gen.	30 mcg	14	18
Cefuroxime 2nd Gen.	30 mcg	14	18
Ceftazidime 3rd Gen.	30 mcg	14	18
Imipenem	10 mcg	13	16
Meropenem	10 mcg	13	16
Doxycycline	30 mcg	12	16
Minocycline	30 mcg	14	19
Nitrofurantoin	300 mcg	14	17

Table No. 2. Susceptibility of *E. coli* against different antibiotics

Sr. No.	Name of antibiotics	Interpretation of result
1	Ampicillin	Resistant
2	Ampicillin / Salbactum	Resistant
3	Amoxycillin / Clavulanic acid	Resistant
4	Piperacillin / Tazobactam	Resistant
5	Cephalothin 1st Generation.	Resistant
6	Cefuroxime 2nd Generation.	Resistant
7	Cefoperazone 3rdGeneration.	Resistant
8	Cefotaxime 3rd Generation.	Resistant
9	Cefixime 3rd Generation.	Resistant
10	Cefepime 4th Generation.	Resistant
11	Imipenem	Susceptible
12	Meropenem	Susceptible
13	Ertapenem	Susceptible
14	Gentamycin	Resistant
15	Amikacin	Susceptible

16	Ofloxacin 2nd Generation.	Resistant
17	Ciprofloxacin 2nd Generation.	Resistant
18	Levofloxacin 3rd Generation.	Resistant
19	Co- Trimoxazole	Resistant
20	Cefoperazone/ Salbactum	Resistant
21	Doxycycline	Resistant
22	Minocycline	Resistant

Out of 22 different antibiotics *E. coli* showed sensitivity against Imipenem, Meropenem, Ertapenem, and Amikacin while it had shown resistance against Ampicillin, Cephalothoin 1st generation, Cefuroxime 2nd generation, Cefoperazone 3rd generation, Ampicillin / Salbactum, Amoxicillin / Clavulanic acid, Piperacillin / Tazobactam, Cefotaxime 3rd generation, Cefixime 3rd generation, Cefepime 4th generation, Gentamycin, Ofloxacin 2nd generation, Ciprofloxacin 2nd generation, Levofloxacin 3rd generation, Co-Trimoxazole, Cefoperazone/ Salbactum, Doxycycline, and Minocycline. This resistance may be due to multiple-drug resistance gene or mutation cause in gene. According to Conan MacDougal (2005), resistance among pathogen isolated in hospital may be linked to antimicrobial use within hospital and within surrounding community. The design of intervention to reduce resistance among particular pathogen in hospital should consider community antimicrobial use as well as hospital use to obtain maximum impact.

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