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RESEARCH ARTICLE

A STUDY ON ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESCHERICHIA COLI ISOLATES FROM EXTRA INTESTINAL INFECTIONS IN A TERTIARY CARE HOSPITAL.

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Abstract

Escherichia coli (**E.coli**), a common human intestinal commensal causes infections in bodily sites outside the gastrointestinal tract and are called Extra-intestinal pathogenic E.coli. ExPEC causes Urinary tract infections, Blood stream infections, Pneumonia, meningitis, bone, skin, and soft tissue infections including both nosocomial and community acquired infections. The increasing trend of developing antibiotic resistance in ExPEC is of global treat which causes increasing morbidity and mortality. As there is no vaccination for ExPEC so it is necessary to analyze the antibiotic susceptibility pattern for empirical treatment in emergency situations. Extended spectrum beta lactamases (ESBLs) hydrolyze β -lactam antibiotics of third generation Cephalosporins, Penicillins and Monobactams. Since the ESBL enzyme genes are usually found in large plasmids, they also contain other antimicrobial resistant genes. AmpC production in E.coli is through plasmids and mutation in their porin structure. Carbapenems are the drug of choice for ESBL producing E.coli but recent time development of resistance is increasingly reported due to production of Carbapenemase. The aim of this study is to test the Antimicrobial susceptibility pattern of Extra-intestinal E.coli isolates. The study was conducted in the department of Microbiology, Stanley Medical College, Chennai during the period October 2018 to May 2019. The institutional ethical committee approval was obtained and clinical samples such as urine, blood, pus, sputum and sterile body fluids were received from 983 patients suspected of bacterial infections. The samples were processed and biochemical test identified 84 E.coli Isolates. Antimicrobial testing, ESBL, AMPC screening and carbapenemase production were tested. E.coli isolates showed resistance to most of the beta lactam antibiotics such as Ampicillin, Cefotaxime and Ceftazidime and also to Ciprofloxacin & Cotrimoxazole,

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Introduction:-

Escherichia coli (**E.coli**), a common human intestinal commensal causes infections in bodily sites outside the gastrointestinal tract and are called Extra-intestinal pathogenic E.coli¹ (ExPEC). ExPEC causes Urinary tract infections, Blood stream infections, Pneumonia, Meningitis, bone, skin, and soft tissue infections including both

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nosocomial and community acquired infections¹. It carries multiple virulence factors that enable to invade and colonize the extra intestinal sites².

The increasing trend of developing antibiotic resistance in ExPEC is of global treat causing morbidity and mortality³. As there is no vaccination for ExPEC, it is necessary to analyze the antibiotic susceptibility pattern for empirical treatment in emergency situations⁴. The mechanism of development of resistance is hydrolysis of antibiotics by Beta-lactamases enzymes produced by bacteria⁵. Extended spectrum beta lactamases (ESBLs) hydrolyze β -lactam antibiotics of third generation Cephalosporins, Penicillins and Monobactams. Since the ESBL enzyme genes are usually found in large plasmids, they also contain other antimicrobial resistant genes. Therefore most ESBL producing organisms are also resistant to Aminoglycosides, Fluoroquinolones, chloramphenicol, and sulfonamides and are multi-drug resistant⁵. ESBL E.coli resistant to Cephalexin is classified as AmpC beta lactamases belong to class C Ambler classification and group 1 by Bush-Jacob's classification⁵. AmpC production in E.coli is through plasmids and mutation in their porin structure⁵. Carbapenems are the drug of choice for ESBL producing E.coli but recent time development of resistance is increasingly reported due to production of Carbapenemase⁶. The genes encoding for carbapenem production commonly detected are KPC, NDM, OXA, VIM, and IMP⁶.

The aim of this study is to test the Antimicrobial susceptibility pattern of Extra- intestinal E.coli isolates. This study was conducted in the department of Microbiology, Stanley Medical College, Chennai, during the period October 2018 to May 2019. The institutional ethical committee approval was obtained and clinical samples such as urine, blood, pus, sputum and sterile body fluids were received from 983 patients suspected of bacterial infection.

Materials and Methods:-

The study was conducted in the department of Microbiology, Stanley Medical College, Chennai during the period October 2018 to May 2019. The institutional ethical committee approval was obtained and clinical samples such as urine, blood, pus, sputum and sterile body fluids were received from 983 patients suspected of bacterial infection. Processing of the sample including gram staining, motility testing and inoculating into Mac-Conkey agar plate, Blood agar plate and CLED in case of urine and incubating at 37°C for 18 to 24 hours were done. The culture showed positivity in 362 samples. Lactose fermenting, Motile, Gram negative bacilli, Catalase positive, oxidase negative, Indole test positive, citrate negative, urease negative, TSI: A/A with gas and absent H₂S, Fermenting glucose, lactose, mannitol, maltose with gas and not fermenting sucrose, Methyl Red positive, Voges-Proskauer test negative were identified as *Escherichia coli*. This included 84 E.coli Isolates and Antimicrobial testing were done modified Kirby-Bauer disk diffusion method as a lawn culture on Mueller-Hinton agar the following drugs:- Ampicillin 10 μ g, Gentamicin 10 μ g, Amikacin 30 μ g, Amoxicillin-clavulanate 20/10 μ g, Piperacillin-tazobactam 100/10 μ g, Cefotaxime 30 μ g, Ceftazidime 30 μ g, Cefazolin 30 μ g (urinary isolates), Aztreonam 30 μ g, Ciprofloxacin 5 μ g, Levofloxacin 5 μ g, Trimethoprim-sulfamethoxazole 1.25/23.75 μ g, Fosfomycin 200 μ g (urinary isolates) and Nitrofurantoin 300 μ g (urinary isolates). Turbidity was compared to a 0.5 MacFarland's Turbidity. The control was prepared by using E. coli ATCC 25922 strains and incubated at 37°C for 18 hours. Inner diameter of the zone of inhibition was measured by using a millimeter scale around each antimicrobial disk was interpreted as sensitive, intermediate or resistant according to the CLSI guidelines of 2018.

ESBL screening

The isolates that were resistant to Ceftazidime (30 μ g) and Cefotaxime (30 μ g) by disc diffusion method with zones of inhibition of $\leq$ 22mm for Ceftazidime and $\leq$ 27mm for Cefotaxime based on the CLSI 2018 guideline were considered as suspected ESBL producers.

Phenotypic Confirmatory Methods for Extended-Spectrum β -Lactamases:

Double disk diffusion method was used to confirm ESBL production by E.coli strains. The test organisms inoculated by lawn culture onto Mueller Hinton agar plate. Ceftazidime (30 μ g) disc vs. Ceftazidime (30 μ g)/ Clavulanic acid (30/10 μ g) disc and Cefotaxime disc vs. Cefotaxime/ clavulanic acid (30/10 μ g) disc were placed at least 20 mm apart, and incubated at 37°C for 18 hours. E.coli isolates demonstrating an increase in zone diameters of more than 5 mm either with Ceftazidime / clavulanic or Cefotaxime/clavulanic-acid were ESBL producers.

Screening for Amp C beta lactamases:

The isolates were screened for AMPC production by testing their susceptibility to Cefoxitin (30 μ g) by Kirby Bauer disk diffusion method. All the isolates with an inhibition zone diameter $<$ 18 mm were labeled as Amp C positive.

Confirmatory test for Amp C beta lactamases

Double disk diffusion method using Cefoxitin (30µg) and Cefoxitin (30µg) + Cloxacillin (200µg) combination were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with test isolates. A zone diameter ≥ 4 mm around the Cefoxitin+ Cloxacillin than the zone diameter around the Cefoxitin disc alone were considered as AmpC producers⁵

Test for Carbapenemase production

The isolates resistant to Carbapenem by disc diffusion method were screened for the production of Carbapenemase. The phenotypic detection of the Carbapenemase production was performed using mCIM and eCIM as per CLSI 2018 guidelines.

Modified Carbapenem inactivation Methods (mCIM)

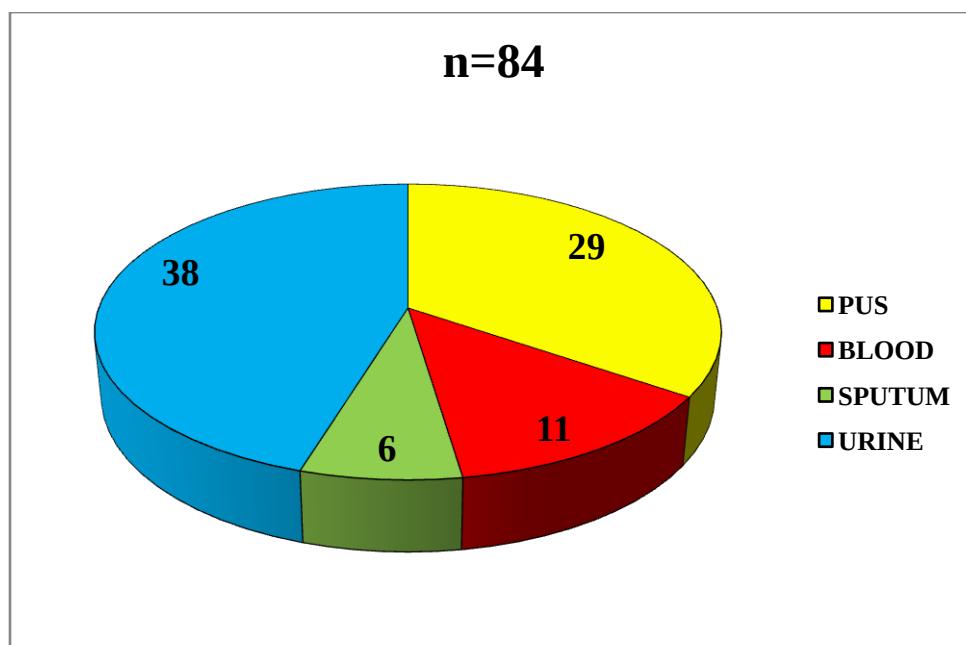
For each isolate 1-µl loopful of pathogen was emulsified and vortexed for 10–15 sec. To this 10-µg Meropenem disk was added to each tube with a sterile forceps. The entire disk is immersed in the suspension. MRP disk was removed from each trypticase soy broth tube and placed on E. coli ATCC 25922 inoculated MHA and incubated.

EDTA Modified Carbapenem inactivation Methods

The same procedure is repeated after adding 20µl of the 0.5 M, EDTA to the 2-mL Trypticase soy broth tube. The MRP disks from the mCIM and eCIM tubes are placed on the same MHA plate, plated with the Meropenem-susceptible E.coliATCC.

Results:-

Pie chart1:- Shows sample wise distribution of ExPECisolates.



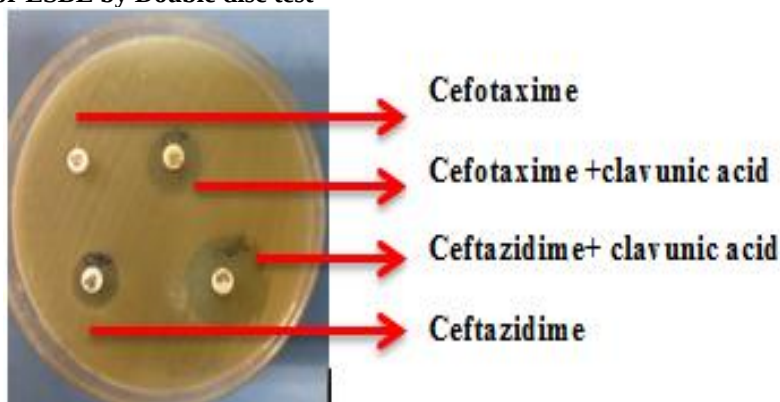
Out of the total 84ExPECisolates, 38 were from urine sample, 29 from pus, 6 from sputum and 11 from blood.

Table 1:- Antibiotic Sensitivity of E.Coli isolated from various samples in percentage.

Sample N=84	Ampicillin	Gentamycin	Amikacin	Amoxicillin-clavulanic acid	Piperacillin-Tazobactam	Cefotaxime	Ciprofloxacin	Levofloxacin	Imipenem	Meropenem	Cotrimoxazole	Aztreonam	Ceftazidime	Fosfomycin	Nitrofurantoin	Cefazolin
Urine% n=38	5	47	74	19	76	18	65	88	97	97	68	47	36	97	92	78
No of isolates	2	18	28	7	28	7	24	33	37	37	26	18	14	37	35	30
Pus n=29 %	6	62	72	41	86	6	14	86	96	93	10	14	10			
No of isolates	2	18	21	12	25	2	4	25	28	27	3	4	3			
Sputum %n=6	16	66	83	66	100	16	33	100	100	100	33	16	16			
No of isolates	1	4	5	4	6	1	2	6	6	6	2	1	1			
Blood% n=11	27	90	100	70	100	27	36	45	100	100	36	36	27	...	...	...
No of isolates	3	10	11	7	11	3	4	5	11	11	4	4	3			

Table 2:- ESBL detection.

ECOLI ISOLATES	ESBL by screening method	ESBL by double disc method	Percentage
Urine n=38	31	20	64%
Pus n=29	4	3	75%
Sputum n=6	5	3	60%
Blood n=11	7	5	71%
TOTAL	47	31	65%

Confirmatory test for ESBL by Double disc test

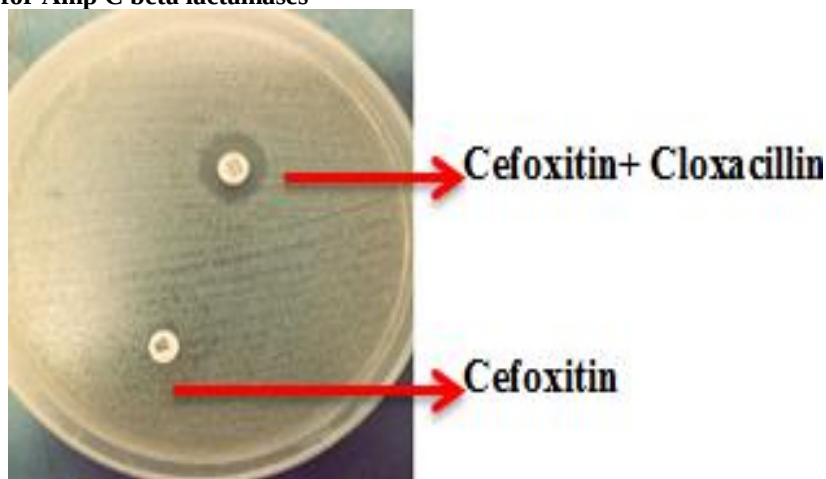
The Picture shows the detection of ESBL by Double disc test using Cefotaxime/ Cefotaxime+ Clavulanic acid and Ceftazidime/ Ceftazidime + clavulanic acid. There is an increase in zone diameter of inhibition of more than 5mm

ESBL detection by chrome agar

The picture shows growth of ESBLs producing magenta coloured EPEC colonies in high chrome agar. All the 31 ESBL isolates detected by double disk test were found to be positive in Hi-chrome agar.

Confirmatory test for Amp C beta lactamases Table 3

ExPEC Isolates n= 73	Screening of AmpC producer by Cefoxitin disc diffusion	Amp C detection by Confirmatory test	
		Total	Percentage
Urine	18	11	61
Pus	13	8	62
Sputum	2	1	50
Blood	3	2	66
TOTAL	36	22	61

Confirmatory test for Amp C beta lactamases

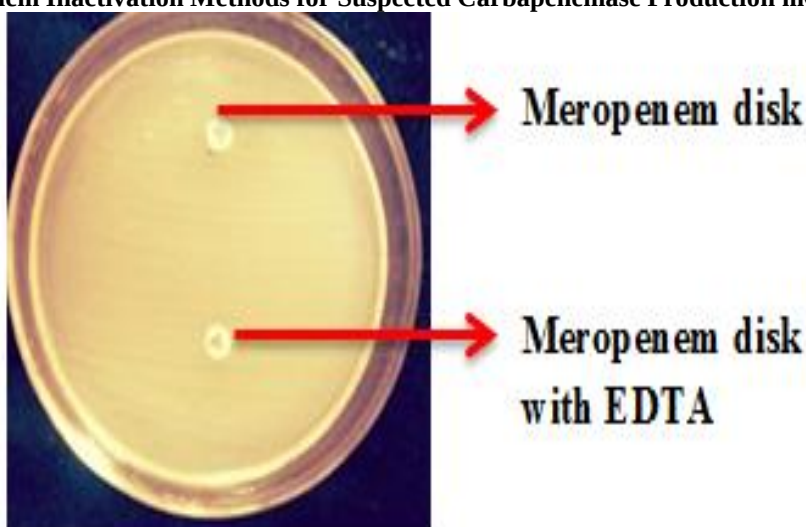
The picture shows AmpC confirmatory test using Cefoxitin and Cefoxitin+ Cloxacillin. There is an increase in zone diameter of inhibition of more than 4mm.

Table 4:- Detection of Carbapenemase producers.

S.No	Total Sample n =2	Screening of Carbapenem resistant by disk diffusion testing	Modified Carbapenem inactivation Methods	
			mCIM	eCIM
1	Pus	1	1	0
2	Urine	1	1	0
3	Sputum	0	0	0
4	Blood	1	1	0
	Total	3	3	0

Shows Carbapenem resistance:-one isolate from pus and one isolate from urine

Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production mCIM and eCIM



Shows Positive mCIM and Negative eCIM

Discussion:-

Isolates with antibiotic resistance are responsible for treatment failure among infected patients. In this study Extra-intestinal E.coli isolates showed resistance to most of the beta lactam antibiotics such as Ampicillin,

Cefotaxime and Ceftazidime and also to Ciprofloxacin & Cotrimoxazole, pattern of various ExPEC isolates were analyzed. A total of 84 ExPEC isolates consisting of 38 isolates from urine, 29 isolates from pus, 6 isolates from sputum and 11 isolates from blood were subjected to antimicrobial testing. It was observed that extra consistent with previous study by Asima Banu, et al.

Among the 84 ExPEC isolates tested, 47 isolates were identified to be resistant to Ceftazidime and Cefotaxime as per ESBL-CLSI 2018 screening test. Of these 22 (30%) isolates were detected to be ESBLs by Double disc test.

Of the 84 isolates, 22 were positive for Amp C beta lactamases by screening test, of these 18 isolates were confirmed AmpC by double disc test. Compared to the study by Sara shayan et al, which showed ESBL producers of 62% and AmpC producer of 5%, but this study shows an increase in AmpC producers as shown in table 2 and 3. This study revealed 2 (2.7%) Carbapenemase producers among extra intestinal *E. coli* isolates, consistent with the previous study by Rituparna Tewari

It is found that most of the beta lactamase producers were also resistant to other groups of antibiotics such as Quinolones and Sulphonamides as shown in table: 1. This is in accordance with the previous study by Asima Banu et al. This can be attributed to ESBL genes that are usually found in large plasmids also contain other antimicrobial resistant genes. Multi drug resistance isolates were 32 (41%). This is comparatively less than to the incidence of 50 % MDR shown in a study by Amhed on ExPEC 2016.

It is noted in chart 4, there is a relatively high antibiotic resistance among the sample received from hospitalized patients compared to outpatients similar to study by Asima Banu et al. The antibiotic resistance among hospitalized patients could be explained because of empirical treatment with multiple antibiotics. But in urinary isolates of extra intestinal *E. coli*, there was high resistance in outpatients similar to study by Asima Banu, et al.

Conclusion:-

The early detection of resistant strains of ExPEC plays a vital role in reduction of morbidity and mortality. Judicious use of antibiotics and a good antibiotic policy are needed to limit the emergence and spread of antibiotic resistance in bacteria. The appropriate selection of antibiotics for the treatment depends on the early and prompt antibiotic sensitivity test. Empirical treatment of antibiotics prior to antibiotic sensitivity report should be avoided unless indicated. Good hand hygiene practices, Health education of healthcare personnel, professionals, contact precautions and minimal use of interventional devices would prevent spread of nosocomial resistant strains.

Conflict of interest:

None.

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