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PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *Escherichia coli* IN A TERTIARY CARE HOSPITAL IN KOLKATA

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ABSTRACT

1.Extended-spectrum β-lactamases

ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem).

1.1 clinical laboratory personnel be concerned about detecting these enzymes because

The presence of an ESBL-producing organism in a clinical infection can result in treatment failure if one of the above classes of drugs is used. ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. Thus, the choice of which antimicrobial agents to test is critical. For example, one enzyme may actively hydrolyze ceftazidime, resulting in ceftazidime minimum inhibitory concentrations (MICs) of 256 µg /ml, but have poor activity on cefotaxime, producing MICs of only 41.1g/ml. If an ESBL is detected, all penicillins, cephalosporins, and aztreonam should be reported as resistant, even if in vitro test results indicate susceptibility [1]

The production of extended-spectrum-beta lactamases (ESBLs) is an important mechanism for resistance to the third-generation cephalosporins. Awareness and the detection of these enzymes are necessary for optimal patient care.

To determine the prevalence and the antibiotic sensitivity pattern of ESBL producing gram negative bacilli, a prospective study was conducted at a tertiary care teaching hospital.

A total of 60 isolates which were recovered between June and August 2011 from various samples were tested for ESBL production by using both the double-disk synergy assay and the combination disk methods.

1.2 Double disc synergy assay-

The double disc synergy test was performed as a standerd disk diffusion assay on Muller-Hinton agar(MHA).0.5McFarland bacterial suspention was inoculated on a MHA plate.The disk containing 30 lig of ceftriaxone was placed 30mm apart around a disc containing 201.tg of amoxicillin plus clavulanic

acid.Enhancement of the inhibition zone between clavulanic acid and test antibiotic,was regarded as presumptive ESBL production[5].

1.3 Combination disc method-

The combination disk method for detecting extended-spectrum f3-lactamases (ESBLs) depends on comparing the inhibition zones of ceftazidime (30μg) and ceftazidime-plus-clavulanate (30/10 μg) disks. The presence of clavulanate enlarged the zones of ESBL-producing ***E.Coli*** *by 5 mm.[2-4].*

1.4 Function of clavulanic acid:-

It has beta-lactam ring,obtained from Streptomyces clavuligerus but no.antibacterial activity of its own.It inhibits a wide varity of beta-lactamases produced by both Gm(+) &Gm(-) bacteria.

I.INTRODUCTION

II.DESCRIPTION

Escherichia coli is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls. [6][7][8] The harmless strains are part of the normal flora of the gut, and can benefit theirhosts by producing vitamin K2, [9] and by preventing the establishment of pathogenic bacteria within the intestine.[10][11] **E. coli** and related bacteria constitute about 0.1% of gut flora, [12] and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination.[13][14] The bacterium can also be grown easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. E. coli is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.

E.Coli can be differentiated from other enteric Gram-negative bacteria by the ability to utilize certain sugars and by a range of other biochemical reaction, such as- indole production and the formation of acid and gas from lactose,take place at 44c° as well as 37c°.

2.1 Biology and biochemistry-

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 , micrometers (μm) long and 5.0. (μm) in diameter, with a cell volume of 0.6 — 0.7 (pm) .[15][16] It can live on a wide variety of substrates. E. coli uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when E. coli lives together with hydrogen-Consuming organisms, such as methanogens or sulphate-reducing bacteria.[17]

Optimal growth of ***E. coli*** occurs at 37°C (98.6°F) but some laboratory strains can multiply at temperatures of up to 49°C (120.2°F) .[18]Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethylsulfoxide and trimethylamine N-oxide. .[19] Strains that possess flagella are motile. The flagella have aperitrichous arrangement. .[20] ***E. coli*** and related bacteria possess the ability to transfer DNA viabacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from ***Shigella*** to ***E. coli*** O157:H7, carried by a bacteriophage. .[21]

2.2 Pathogenecity-

The adhesion might be involved in pathogenecity.eg-filamentous protein structure resembling fimbriae cause mannose resistant haemagglutination and there is a good evidence, that play an important role in pathogenesis of diarrhoeal disease and in urinary tract infection and other infection.The colonizing factor antigens (CFAS) expressed by exterotoxigenic E.Coli that cause human diarrhea disease.

In the absence of acquired resistance E.Coli is susceptible to many antibacterial agents including-ampicillin,cephalosporins,tetracyclins,quinolones.Some strains of E.Coli can produce- Extended Spectrum Of Beta-Lactamases, and are responsible for their resistance to beta lactam antibiotics. These antibiotics have a common element in their molecular structure, a 4 atom ring know as- beta lactam.The lactamases enzyme breaks that ring open, deactivating the antibacterial properties. Different beta-lactamases differ in their substrate affinities.2 inhibitor of this enzyme-clavulanic acid & sulbactam are available in clinical use.

2.3 The classification of Extended sprectum of beta latamases:

- TEM beta-lactamases (class A) .
- SHV beta-lactamases (class A) .
- CTX-M beta-lactamases (class A)
- OXA beta-lactamases (class D)

Detection of ESBL, is a major challenge for the clinical microbiology lal-)oratory,as its detection has major impact on therapy,Moreover, prescence of an ESBL also has significant infection control implication .the resistance to extended spectrum cephalosporins is mainly mediated by the production of ESBLs [22]. A number of nosocom ial outbreaks which were caused by ESBL-producing organisms, have been reported in the UnitedStates[23-25]

Therefore, the threat a these resistant organisms is not limited to intensive care units or tertiary hospitals.

Recent studies on ESBL, production among the members of Enterobacteriaceae which were isolated from clinical specimens, showed an increase in the occurrence of ESBI, producers [26] st .udti from North India on uropathogens such as ***Klebsiella pneumoniae, Escherichia coli, Enterobacter,, Proteus and Citrobacter*** spp., show ed that 26.6% of the isolates were ESBL producers [27] A study from Nagpur showed that 48.3% of their celota i me resistant gram negative bacilli were ES131, producers [28] A report from Coimbatore (India) showed that ESBL production as 41% in E. coil [29]

In a similar study by Mathur et al, 62% of the E. coli and 73% of the K. pneumoniae isolates were reported to be ESBL producers [30]

2.5 Inclusion Criteria:-

1. Patients attending the outdoors of CSTM.
2. Patients admitted in the wards of CSTM.
3. Urine, blood, pus, wound swab and any body fluid samples.
4. Pure isolates of ***E.coli.***
5. Antibiotic sensitivity zone diameters to be correlated according to CLSI guideline.

2.6 Exclusion Criteria:-

1. Patients inadequately treated with antibiotics.
2. Grossly contaminated growths.
3. Stool samples.

2.7 Risk factors for community acquired infection by ESBL

- Recurrent urinary tract infection. slat
- Diabetes mellitus.
- Previous antibiotic usage.
- Prior instrumentation to urinary tract.
- Female sex.
- Age (over 50 years).
- Prior hospital admission.
- Immuno-compromised patient.
- Structural anomaly of Urinary tract.

III.AIM

To determine the prevalence of ESBL producing ***Escherichi Coli*** strains isolated from urine and pus sample of hospitalized and non hospitalized patients to determine antibiotic susceptibility.

IV.OBJECTIVE

To assess,

- The clinical features
- Risk factors
- Prevalence
- Outcome of extended sprectum beta lactamase producing
- ***Escherichia. Coli*** infection in hospitalized and non-hospitalized patient.

V.MATERIALS & METHODS

such as **pus, sputum, cerebrospinal fluid, ascitic fluid,pleural fluid, blood and urine** will included in our study. The isolates will identify, based on the standard bacteriological techniques and will tested for ESBL production by using –

A prospective study will done on different types of samples of hospitalized & non hospitalized patients.

Specimen

The double-disk synergy assay test which was described byJarlier et al .

&

Combination disc method was recommended by**CLSI** guidelines. [31-36]

Extended sprectum of beta lactamase (ESBL) producing***Escherichia.Coli*** have been increasingly recognized in Kolkata.The aim of this study was to determine the prevalenceacquired of ESBL producing ***E.coli,*** in hospitalized patients and Community acquired infection.

5.1 Materials

The materials that were required and used for detection of extended spectrum of beta lactamase producing ***E.coli*** are below,

5.1.1. Glassware:-

a. Sterilized test tubes

b. Grease free slides

5.1.2. Sample:-

a. Urine

b. Pus

c. Blood

d. Body fluids

5.1.3. Media:-

a. Mueller Hinton Agar media

b. Mac Conkey's Agar media

c. Blood Agar media

d. Peptone water

e. Normal saline.

5.1.4..For Biochemical Test

a. Kovac’s reagent

b. Methyle red indicator

c. Triple sugar iron agar

d. Simmon Citrate media

e. Gelatin

f . Urea media

g. Tryptophane broth

5.2 Methods

At first samples were collected from patients.

With the help of inoculationloop,a loopful of urine was streaked on both Mac Conkey and blood agar.Swabstics were used for collection of pus from patients.

Total procedure was done under strict aseptic condition to prevent contamination.

Then the plates were incubated overnight in an incubator at 37c ° (which is the optimum temperature for growth of microorganisms)

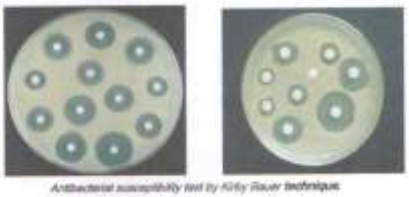
After 24hr the growing colonies on plates were stained by Gram's staining method,to characterize organism's morphology.

5.2.1 Identification of bacterial pathogens

Preliminary identification of bacteria was based on colony characteristics of the organisms, changes in physical appearance on differential media & enzyme activities of the organisms .Biochemical tests were performed on colonies from primary cultures for identifications of the isolates

5.3Antibacterial susceptibility testing

- The susceptibility testing will performed by Kirby Bauer technique.
- The test organism will uniformly seeded over the Mueller- Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic-impregnated paper disc into the agar medium.
- the isolates will then incubated at 37c °for 18hr.
- Organism sensitive to the antibiotic will inhibit from growing a zone of inhibition will observed around the antibiotic impregnated paper disc.
- Grades of sensitivity recognized are-sensitive, intermediate and resistant by comparison of zone of inhibition as indicated (CLSI).
- The drugs are tested againsted the Gram-negative rods were-gentamycin(10µg),ceftazidime(30µg ampicillin(30 µg), meropenem, amikacin, nitrofuranton, levofloxacin, methecillin, ceftriaxone.
- Those organisms resistant to ceftazidime were further examined for double disc synergy assay and combination disc method to detect whether the organisms were ESBL producer or not.



Antibacterial susceptibility test by Kirby Bauer technique.

5.3.1 Disc Synergy Method

5.3.2 Inoculums preparation:

The inoculum will prepared by picking the test organisms with a sterile wire loop.

These was suspended in sterile peptone water. then inoculated upto 4 hr,allow organisms reach their log phase in growth.

The density of suspension to be inoculated was determined by comparison with opacity standard on Mc Farland 0.5 Barium sulphate solution.

5.3.3 Antibiotic sensitivity test:

A sterile cotton swab was dipped into the suspension of the isolate in peptone water, squeezed free from excess fluid against the side of bottle and then spread over the surface of Mueller Hinton agar plate (Mueller Hinton agar is drug sensitive media).

5.3.4 Double disc synergy assay:

In double disc synergy test the disk of ceftriaxone will placed 30mm apart around a disc of amoxicillin plus clavulanic acid. Enhancement of the inhibition zone between clavulanic acid and test antibiotic: will regard as presumptive ESBL production.

5.3.5 Combination disc method:

In combination disk method for detecting (ESBLs) depends on comparing the inhibition zones of ceftazidime(30µg) and ceftazidime-plus-clavulanate (30/10 µg) disks. The presence of clavulanate enlarged the zones of ESBL-producing ***E.Coli*** by ≥ 5 mm.

Thus ESBL producing ***E.coli*** were ensured by both- **Double disc synergy assay** and **Combination disc method** according to the Clinical Laboratory standards Institute Guidelines (CLSI).

VI.CONCLUSION

- From this study such resistant strain analysis will allow a better understanding of the dynamics and function of the urine &,blood, associated microbiota. It will be possible to explore bacterial communities in an unprecedented way by highlighting metabolically active bacteria and discovering novel bacterial genes that play important roles in chemical and biological processes of the human body.
- As microbial constrain adaptation to environmental fluctuations, better knowledge of blood & urine - associated bacterial communities will be an important aspect of understanding what drives ***Escherichia coli*** producing ESBL adaptation.
- The overall study focuses many important aspects of this strain. It might be concluded that owing to the presence of diversified beneficial features if displayed by this strain, it might be a candidate that links to urinary tract infection and spread can be ascertaine
- The ESBL-producing organisms are a breed of multidrug - resistant pathogens.
- It is increasing rapidly and becoming a major problem in the area of infectious diseases.
- It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing proper antibiotics.

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