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RESEARCH ARTICLE

CHANGING TREND OF ANTIMICROBIAL RESISTANCE PATTERN IN *ESCHERICHIA COLI* CAUSING URINARY TRACT INFECTION AMONG HOSPITALIZED PATIENTS

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ABSTRACT

Urinary tract infections (UTI) are one of the commonly encountered diseases in developing countries. This study determines the antibiotic resistance patterns of *E.coli* from Hospitalized UTI patients. Study was carried out in one year period. Among the *E.coli* isolates the resistance pattern was studied using the antimicrobial susceptibility pattern and using advanced expert system of Vitek 2 compact system. The resistant profile was analysed for Beta-lactam phenotypes and Amino glycosides phenotypes. In the Beta lactam phenotypes 62% of the isolates revealed ESBL and 8% of the isolates as Carbapenamase producers. Whereas in Amino glycosides phenotypes 35% accounts for *aac (3)* resistance and 50% of the isolates are wild types. This study was determined to understand the epidemiological resistant patterns in the isolated strains in the hospitalized patients. Antibiotic stewardship is becoming the growing trend in the health care systems to restrict the spread of multidrug resistant strain and to avoid the empirical treatment. This specific categorised statistical analysis will enhance the adherence of antibiotic policyin adult in with typical presentation.

Key words: *E.coli*, UTI, resistant, Beta-lactam, Aminoglycoside.

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INTRODUCTION

Urinary tract infections (UTI) are one of the commonly encountered diseases in developing countries. UTI can be caused by Gram-Negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Proteus* species and Gram positive bacteria like *Enterococcus* species and *Staphylococcus saprophyticus*. *E.coli* is the most common organism causing both community as well as hospital acquired UTI. The drug of choice for treating *E.coli* infections are becoming limited due to the rise in antibiotic resistance. Antibiotics are used to treat UTI. Over time, however, many bacteria have become resistant to antibiotics. Antibiotic resistance is a serious problem for individual patients and healthcare systems; in hospitals, infections caused by antibiotic- resistant bacteria are associated with higher rates of death. The main objective of the study is to provide convincing evidence and information to educate and support professionals to reduce unnecessary use and minimize the increased risk of infection with antibiotic –resistant bacteria. In this study, the objective was to analyse the antimicrobial resistance patterns of collected *E.coli* strains. The resistant pattern was analysed based on the phenotypic susceptibility by analysing the resistance extended by MIC values and by the automated interpretation of Advanced Expert System (AES) reported by Vitek 2 system[10]. AES is based upon an extensive knowledge base that comprises over 2000 phenotypes and 20,000 MIC distributions[3]. A phenotype is defined as the expression of a specific mechanism of susceptibility or resistance to a given drug class within a particular species. There are number of possible phenotypes are considered under each group of antibiotics. In this study a detailed phenotype analysis was carried out for β-lactam drugs and Aminogly cosides drugs for the selected *E.coli* strains. This statistical data analysis was carried out to know the epidemiological resistant pattern for about a period of one year.

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MATERIALS AND METHODS

Specimen collection: A total of 7128 urine samples were received during the study period from March 2016 to February 2017. All the samples were collected in the sterile container with the instructions "Clean catch mid-stream urine". Urine culture was done by standard loop method, a semi-quantitative method in Blood agar and Chrom UTI agar. Colonies grown 10^5 and above are considered as significant and taken for further follow-up. These samples were also compared with the microscopy of the specimen.

Strains: A total of 7128 urine samples were collected in a period of about one year from March 2016 to February 2017. Among that 397 isolated *E.coli*strains were taken for the analysis study. Their beta lactam phenotypes, aminoglycosides phenotypes were characterized by biochemical and molecular techniques.

Identification: The organism was identified preliminary by the colour morphology in HiChrom UTI agar (MP1353 from Biomeriux). The organisms isolated from urine werethen identified by Vitek 2 system (GN-ID). The antibiotic susceptibility test was also carried out by Vitek 2 system (AST-N281).

Susceptibility tests: Antibiotic susceptibilities were determined according to the manufacturer's recommendations by using the Vitek 2 instrument. The card used (AST-N281) for the test contained the following antibiotics and concentration ranges: Amikacin 2 to 64 µg/ml; Aztreonam 1 to 64 µg/ml; Cefepime 1 to 64 µg/ml; Cefoperazone/Sulbactam 8 to 64 µg/ml; Ceftazidime 1 to 64 µg/ml; Ciprofloxacin 0.25 to 4 µg/ml; Colistin 0.5 to 16 µg/ml; Doripenem 0.12 to 8 µg/ml; Gentamicin 1 to 16 µg/ml; Imipenem 0.25 to 16 µg/ml; Levofloxacin 0.12 to 8 µg/ml; Meropenem 0.25 to 16 µg/ml; Minocycline 1 to 16 µg/ml; Piperacillin/Tazobactum 4/4 to 128/4 µg/ml; Ticarcillin/Clavulanic acid 8/2 to 128/2 µg/ml; Tigecycline 0.5 to 8 µg/ml; Trimethoprim/Sulfamethoxazole 20(1/19) to 320(16/304) µg/ml. Quality control was performed as per kit manufacturer's instructions with *E.coli* ATCC 25922; *P.aeruginosa* ATCC 27853; *E.coli* ATCC 35218 monthly and whenever new lot received.

Data analysis: Both the identification and susceptibility was done using Vitek 2 system. The results were analysed and statistics made. The resistant phenotype identified by the AES was compared with the resistant profile of the antibiotics, and manual methods like Disc diffusion test for ESBL and Modified Hodge test for Carbapenamase producer strains.

RESULTS

Out of 7128 urine sample received, 1306 (18.32%) samples showed significant positive growth. It includes 1058 (81.01%) Gram negative bacilli, 138(10.56%) Gram positive cocci and 110 (8.42%) Yeast like cells. Among the 1058 Gram negative bacilli *E.coli* was identified in 674 patients with 397 IP and 277 OP isolates. The IP had 227 female and 170 male patients. The Age category include <20 = 3 patients; 20 to 40 = 41 patients; 41to60 = 105 patients and >60 = 248 patients. The resistant phenotypes was analysed for these 397 isolates.

Species: The preliminary identification of the organisms was done by the Hi ChromUTI agar. (Table. 1) The final identification and AST was confirmed by VITEK 2 system. The susceptibility pattern was confirmed with the MIC values of each antibiotic.

Phenotype Analysis

The performance of AES for the species is analysed to identify resistant phenotype. The AES was analysed for the Aminoglycoside phenotype and β lactam phenotype.

Beta lactams: The beta lactam phenotypes was categorised into 12 types including wild type (Fig.1) The brief explanation and the statistical data are below (Table 2)

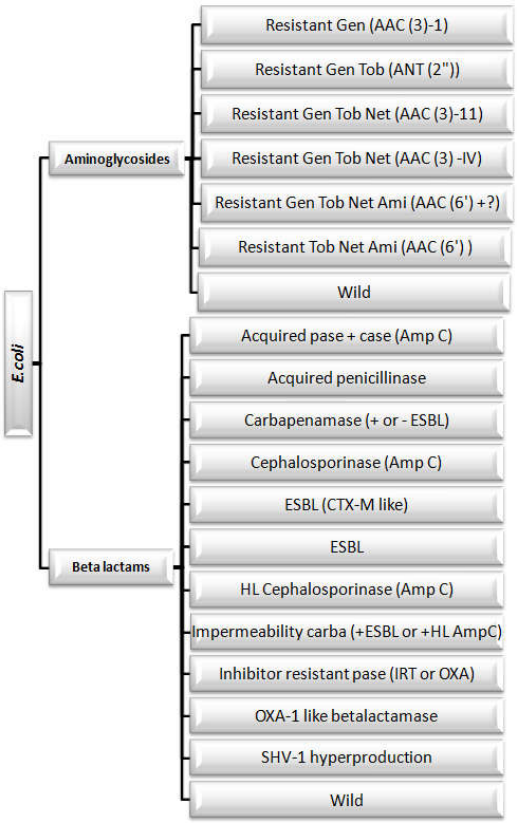

Fig. 1. Dendogram showing the beta lactam and aminoglycoside phenotypes

Table 1. Colour identification of gram negative bacilli in hichrom uti agar

Gram Negatives	Colour in HiChrom UTI Agar
E.coli	Pink centred cream bordered colonies flat irregular colonies
Klebsiella	Green mucoid, raised, round colonies
Enterobacter/ Citrobacter	Dark green flat irregular colonies
Pseudomonas	Pale Brown dirty cream flat irregular colonies
NFGNB	Cream fine round colonies
Proteus	Cream flat flowery colonies

Aminoglycosides: According to Cathrine Barnhart *etal*. 2002, there are three mechanisms of amino glycoside resistance: reduced uptake or decreased cell permeability, alterations at the ribosomal binding sites, or production of amino glycoside modifying enzymes. The enzyme modification is the most common type of amino glycoside resistance which results in high level resistance. These genes are usually found on plasmids and transposes. Most enzyme-mediated resistance in gram negative bacilli is due to multiple genes. The three types of amino glycoside modifying enzymes are:

Table 2. Beta Lactam Phenotypes

Types	Phenotype	Description	Reference	Percentage
1	Acquired pase + case (AmpC)	Amp C enzymes are encoded by both chromosomal and plasmid genes. Transmissible plasmids has acquired genes for AmpC enzymes, resistance due to plasmid – mediated AmpC enzymes are less common	Jacoby, 2009	29.72%
2	Acquired Penicillinase	Penicillins and certain older cephalosporins like cephalothin are the affected drugs. Examples of Beta lactamases are TEM-1, SHV-1, OXA-1.	Christine *et al.,* 2000	28.96%
3	Carbapenamase (+ or – ESBL)	Carbapenem resistant due to presence of Carbapenamase with ESBL or without ESBL	ECDC, 2016	8.31%
4	Cephalosporinase (Amp C)	Same as ESBL plus inhibitor-drug combinations and cephamycins. Plasmid – mediated AmpC, hyperproducers of Chromosomal AmpC are the examples.	Christine *et al.,* 2000	28.90%
5	ESBL (CTX-M like)	ESBL with the prominent presence of CTX-M family of enzymes which hydrolyse cefotaxime more efficiently than ceftazidime	James *et al.,* 2010	29.72%
6	ESBL	Same as acquired penicillinase plus newer cephalosporin's and aztreonam. Examples of Beta lactamases are TEM-3, SHV-2	Christine *et al.,* 2000	61.46%
7	HL Cephalosporinase (AmpC)	The phenotype results from mutation in regulatory genes that control the amount of the basal level of AmpC β-lactamase and the inducibility of enzyme expression	Christine *et al.,* 2000	33.75%
8	Impermeability Carba (+ESBL or + HL AmpC)	Same as HL Cephalosporinase plus reduced susceptibility to Imipenem, Meropenem as well as other β-lactam Antibiotics	Christine *et al.,* 2000 and based on our statistical results	7.30%
9	Inhibitor Resistant Pase (IRT or OXA)	The phenotypes results in Amoxycillin – Clavulanate resistance due to the presence of OXA-1 and inhibitor resistant TEM (IRT) β-lactamases	Oteo *et al.,* 2014	29.47%
10	OXA-1 like beta lactamase	? Identification of the phenotype seen in a single isolate.	Our statistical results	0.25%
11	SHV-1 Hyperproduction	The phenotype mutates to produce as ESBL derivative	Christine *et al.,* 2000	0.50%
12	Wild	The wild type of this species produces no significant level β-lactamase	Christine *et al.,* 2000	27.45%

Table 3. Aminoglycosides phenotypes (Christine C. Sanders *et al*., 2000)

Types	Phenotype	Genes	Selected Aminoglycoside substrates	Percentage of the isolates
	Acetylation			
1	Resistant Gen (AAC (3)-1)	*aac(3)-Ia* *aac(3)-Ib*	Gen	22.92%
2	Resistant Gen Tob Net (AAC (3)-II)	*aac(3)-Iia* *aac(3)-Iib* *aac(3)-Iic*	Gen, Tob	22.92%
3	Resistant Gen Tob Net (AAC (3)-IV)	*aac(3)-Iva*	Gen, Tob	22.92%
4	Resistant Gen Tob Net Ami (AAC (6') +?)	*aac(3)-Via*	Gen, Tob	34.49%
5	Resistant Tob Net Ami (AAC (6'))	*aac(6')-aph(2")*	Gen, Tob, Ami	21.91%
	Adenylylation			
1	Resistant Gen Tob (ANT (2"))	*ant(2")-Ia* *ant(2")-Ib* *ant(2")-Ic*	Gen, Tob	22.92%
1	Wild		No specific mutations	49.62%

1. N-Acetyltransferases (AAC) – catalyzes acetyl CoA-dependent acetylation of an amino group
2. O-Adenyltransferases (ANT) – catalyzes ATP – dependent adenylation of hydroxyl group
3. O-Phosphotransferases (APH) – catalyzes ATP – dependent phosphorylation of a hydroxyl group.

The aminoglycosides phenotypes was categorised into 7 types including wild type (Fig. 1). The brief explanation and the statistical data are below (Table 3)

DISCUSSION

A total of 397urine was analysed for the resistant profile using Vitek 2 AES for β-lactam phenotypes and Amino glycosides phenotypes. The β-lactam wild type and Amino glycosides wild type was seen in 27.45% and 49.62% respectively, which means prior to any mutation of chromosomal genes or acquisition of new DNA that alters susceptibility to the drug class in question. In other words the wild type of β-lactams phenotypes produces no significant level of β-lactamases; the wild type amino glycoside produces no enzymatic modification. Among the β-lactam phenotypes Type 6 (ESBL) was recorded as 61.46% which was much higher that any of the other phenotypes. These strains are resistant to any one of the third generation cephalosporins, Aztreonam. The beta lactam/beta lactam inhibitors are sensitive. At the same time our resistance is much lesser when compared with other Indian studies like Niranjan & Malini (2014) reported 70%; and Sharma *et al.* (2016) 73%. The multiple phenotypes of β-lactams were recorded in 3 different patterns.

Penicillin's and Cephalosporin's resistance: The combined phenotypes are Type 1, 2, 4 and 5 which showed up to 27.70%. As described earlier and by various authors these strains extends the resistance to Penicillis, older and newer cephalosporins, aztreonams, plus inhibitor drug combinations, cephamycins, including Plasmid – mediated AmpC, hyperproducers of Chromosomal Amp C.

High level Cephalosporins

The combined phenotypes are Type 6 and 7 recorded in 33.75%. These strains showed high level resistance to 3rd

generation cephalosporins with increased MIC values and partially resistant to 4th generation also.

Carbapenamase resistance

Type 3 Carbapenamase (+ or – ESBL), Type 8 Impermeability carba (+ESBL or HL Amp C) and Type 11 n with 8.31%, 7.30% and 0.50% respectively. The high-level resistance to carbapenems by such carbapenamases is essential of three types – KPC, MBLs and Oxacillinases. Carbapenamases are beta lactamases and by tradition the nomenclature of the beta lactamases is based on their substrates, biochemical properties, location of their discovery, location of the gene on the chromosome, strains of bacteria, patients providing the sample or even after the investigator who describe them (Camillia, 2011). The multiple phenotypes of Amino glycosides were recorded in a pattern. Which include Type I, 2, 3, 4 and 5 in 14.60% and Type 1, 2,3 and 4 in 22.92%. All these strains show acetylation with extension of resistance from single amino glycoside to multiple antibiotics. Many patients who get antibiotics for UTIs actually have asymptomatic bacteraemia and not infections. Interventions for UTIs focus on avoiding unnecessary urine cultures and treatment of patients who are asymptomatic and ensuring that patients receive appropriate therapy based on local susceptibilities and for the recommended duration. These recommendations are possible only if the supportive professionals are educated with the categorised resistant patterns. These should be updated periodically. Reporting the resistant pattern with AES will help the clinician to understand resistant pattern with probable mutation of the genes. But in the present study the phenotypes are not confirmed with the molecular analysis, it only shows the exact data analysis with the help of susceptibility pattern. The description of the phenotype was analysed with the previous research studies and articles. However the AES was very useful for the identification of the phenotypes of gram negative isolates. The multiple phenotype resistance in *E.coli* strains could be due to the high prevalence of resistant strains in the community. But the percentage of resistant is less due to the strict infection control practices and adherence to standard precautions.

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