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Urinary Tract Infection and Antimicrobial Susceptibility Pattern of Extended Spectrum of Beta Lactamase Producing Clinical Isolates

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Abstract: Retrospective analysis was done on the resistance pattern of urinary tract pathogens isolated over a month's period. Total of 871 clinical isolates comprising of 793 gram negative bacilli and 78 gram positive cocci were obtained from 6405 consecutive urine samples. Extended spectrum beta lactamase (ESBL) production was observed in 71.5% of gram-negative bacilli. Of these 6.18% were also inhibitor resistant. 70.17% of *Enterococcus* isolates were resistant for high levels of aminoglycosides, ciprofloxacin. Multidrug resistance and ESBL is a common problem in hospital which emphasizes the need for judicious use of antimicrobial agents and their continuous *in vitro* monitoring.

Key words: ESBL % Antibiotic susceptibility % Beta Lactamase % Cefotaxime % UTI

INTRODUCTION

Extended Spectrum of β-lactamase (ESBL) producing bacteria in recent years pose critical problems for the clinical microbiologist and physicians. Urinary tract infections (UTIs) is one of the most common infectious diseases ranking next to upper respiratory tract infection is an important cause of morbidity and mortality in human. Infected urine stimulates an immunological and inflammatory response leading to renal injury and scarring, ultimately leading to end stage renal failure. Renal calculi, obstructive uropathy (posterior urethral valves), vesico ureteral reflux and avoiding disorders can lead to urinary stasis and may predispose to the development of recurrent UTI and complications [1]. It has been estimated that nearly 10% of the human population will experience a UTI during their life time [2-4]. Bacteria are the major causative organisms and are responsible for more than 95% of UTI cases, *Escherichia coli* is the most prevalent causative organisms of UTI and is solely responsible for more than 80% of the infections [5,1]. Treatment of UTI cases is often started empirically. Therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. However, because of the evolving and continuing antibiotic resistance phenomenon, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy [1,6,7]. ESBLs are enzymes that inactivates third generation cephalosporins (ex. ceftazidime, cefotaxime and cefepime) and monobactam (ex, aztreonam,) and are inactivated by Clavulanic acid [8]. The presence of an ESBL producing strains in severe infections can result in the failure of the treatment [9]. May be the prevalence and antimicrobial resistance pattern may vary between geographical areas. The aim of this study was to determine the causative agents of UTI and their susceptibility patterns to commonly used antibiotics in South west Tamil Nadu.

MATERIALS AND METHODS

Study Design: The study was carried out in the Department of Microbiology, Bharathidasan University, Tiruchirappalli and collaborated with Miritasanjivini Lab Pvt Ltd Coimbatore. Retrospective analysis were done on six thousand four hundred and five patients who were diagnosed as positive for urinary tract infection within the ages 0 to 60 among both male and female and both outpatients and inpatients. Further details for organisms grown in urine culture and their antimicrobial sensitivity

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pattern for the past 13 months (September 2006 to September 2007) were analysed using non probability sampling technique. A detailed history was taken and complete clinical examination was carried out for each case of UTI. Each and every patients had urinary microscopy, urinary colony count and urine culture investigation. The diagnosis of urinary tract infection was based on microscopic findings of more than 5 white blood cells per high power field on urine microscopy and a colony count 10^5/ml of single pathogen. The adult patients were sampled by clean catch midstream urine and in case of neonates the urine was collected through suprapubic approach and in the children aged less than 3 years were sampled using sterile urine bags. All the antibiotics were discontinued 72 hours before sending the urine culture and sensitivity. Urine samples were delivered to the laboratory within 1 h of collection and processed within 2–4 hours of the collection.

Isolation and Identification: All the collected samples were inoculated on blood agar and MacKonkey agar and incubated at 37°C for 24 h and extended to 48 h in negative cases. Bacterial identification was done based on standard bacteriological culture and biochemical characteristics of the isolates [5,1]. Where multiple growths were obtained the culture was repeated again before accepting the results.

Antimicrobial Susceptibilities of Gram Negative Bacterium: Antimicrobial susceptibility of the isolates was tested by the disc diffusion method according to the Bauer *et al*., [10]. The inoculum adjusted to turbidity of 0.5 at OD_{620nm} was swabbed on to Muller Hinton agar plates. The commercial antibiotics used for *E.coli* isolates includes (µg/disk) ampicillin (10);amikacin (30), gentamycin(10),tobramycin(10) ciprofloxacin(5) co-trimoxazole (trimethoprim-sulfamethaxozole, (1.2 / 23.8),tetracycline (30),cefotaxime (30) and ceftriaxone (30) imipenem (10). The resistant pattern of *Klebsiella pneumoniae* for all the above mentioned antibiotics with nitrofurantoin (300) and norfloxacin (10) were also studied. The results were interpreted according to NCCLS 2000.

ESBL Detection by NCCLS Phenotypic Method: The NCCLS ESBL phenotypic confirmatory test with ceftazidime (CAZ) was used for all the gram negative isolates by the disc diffusion method [11]. Muller- Hinton agar plates and disks containing 30µg of ceftazidime with and without 10µg of clavulanic acid (CA) were used. Susceptibilities test results were interpreted according to the NCCLS = 5 mm enhanced in the zone diameter of CAZ versus its zone when tested alone was considered indicative of ESBL production. We have not done the routine test for the presence of ESBL or carbapenamses. However resistance to the third generation cephalosporins is highly suggestive of the presence of ESBLs in *E.coli* and *Klebsiella pneumoniae* [12]. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* 700603 were used as a control strains. The group of isolates where zone of inhibition around both the disks was absent were interpreted as inhibitor resistance.

RESULTS

Clinical Strains Isolated: For the thirteen month of study period,6405 urine samples were received from clinical laboratory and cultured, among the culture screened for significant bacteriuria was found in 1084 (16.3%) samples, where 5321(83.07%) samples were observed to be culture negative. In 287(4.48%) samples there was insignificant bacteriuria. Diptheroids are environmental bacillus species were isolated in 29(0.4%) samples. These 29 samples were not processed further and not included in this study. (Table 1) Among the 1084 culture positive samples, the number of bacterial pathogens obtained were 1084 (1055 had a single pathogen and 29 has 2 types of bacteria grown on culture). The 1084 isolates have been identified as *E.coli* (240, 30.26%), *Klebsiella* sp (175, 22.06%), *Pseudomonas* sp (98, 12.35%), *Acinetobacter* (66, 8.32%), *Proteus* sp (49, 6.3%), *Enterococcus* (76, 9.58), *Staphylococcus* sp (40, 5%), *Enterobacter* (28, 3.5%), *Citrobacter* sp, (20, 2.5%), *Serratia* spp (1, 0.12%).

Table 1: Clinical strains isolated from the Urine samples from September 2006 to September 2007

S.No	Organisms isolated	No(n)	Percentage of isolation	ESBL production % out of 793
1.	*Escherichia coli*	240	30.26%	60.7%
2.	*Klebsiella spp*	175	22.06%	78.7%
3.	*Pseudomonas spp*	98	12.35%	84.67%
4.	*Acinetobacter spp*	66	8.32%	95.23%
5.	*Proteus spp*	49	6.7%	69.44%
6.	*Enterococcus spp*	76	9.58%	-
7.	*Staphylococcus spp*	40	5%	-
8.	*Enterobacter spp*	23	3.5%	70%
9.	*Citrobacter spp*	20	2.5%	94.73%
10.	*Serratia spp*	1	0.12%	-

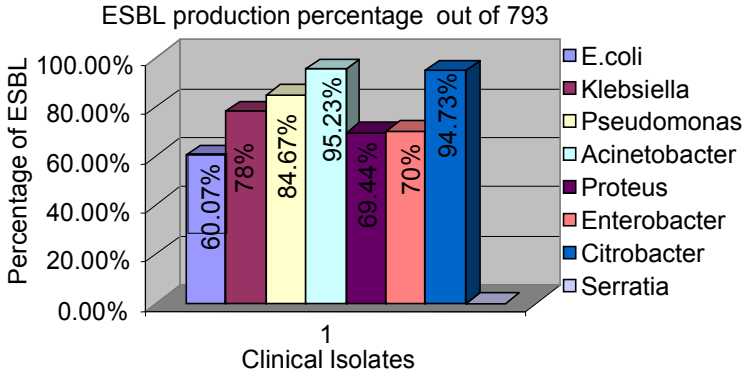

Chart 1: ESBL production percentage put of 793

In 63 samples polymicrobial infection was found the combination of *Klebsiella* + *Pseudomonas* (41 sample) was commonest followed by *E.coli* + *Klebsiella* (22 sample).

Antimicrobial Susceptibilities of Gram Negative Bacterium: Among the 793 gram negative bacterial isolates, only 5.92% (47 out of 793) were sensitive to all antibiotics tested. Multidrug resistance were observed in 90% (714 out of 793) of the isolates. Although 71.5% (567 out of 793) were ESBL producers (Chart 1); and 6.18% (49 out of 793) were also inhibitor resistant. Extended Spectrum Beta Lactamase production ranged from 60.7% (lowest in *E.coli*) to 95.23% (Highest in *Acinetobacter* spp) in case other organisms, ESBL production were as follows; *Proteus* (69.44%), *Enterobacter* (70%), *Klebsiella* (78.7%), *Pseudomonas* (84.67%) and *Citrobacter* (94.73%). Inhibitor resistance was maximum in *Pseudomonas* species. With regards to piperacillin, lowest resistance was observed for *Pseudomonas* species (resistance rate 59.7%) as compared to other gram negative bacteria (resistance rate 78.32%) reinforcing its antipseudomonal effect. ampicillin resistance was observed in 75% (600 out of 793) and ciprofloxacillin resistance was observed in 77.5% (619/793). Among the aminoglycosides, Amikacin showed the best activity (resistance rate 59.5%) as compared to tobramycin (resistance rate 81.31%) and netilmicin (resistance rate 60.39%). Overall, the gram negative bacilli (except *Pseudomonas*) were most susceptible to nitrofurantoin (resistance rate 55.75%).

DISCUSSION

Multidrug resistance and ESBL producing gram negative bacteria are the major cause of infection to the urinary tract. The prevalence of ESBLs among clinical isolates much varies to globally and in various geographic regions and are rapidly changing over time [13]. In this study 6405 patients who were diagnosed as victims of UTI were sampled. Only 16.3% had infected by UTI. As per in our study, most of the urine samples were collected from patients who did not have a combination of UTI symptoms and all the patients were referred by general physician. These findings indicated that urine culture is necessary for a definitive diagnosis of UTI [14]. Extended spectrum of beta lactamase (ESBL) production was noted in more than 70% of all gram negative bacteria. With the highest incidence in *Acinetobacter* species (95.23. %) and *Citrobacter* species (94.73%). Ciprofloxacin resistance was observed in 77.5% (619/793) of the isolates. Babypadmini and Appalaraju, [15] have studied; the occurrence of ESBL producers in urinary isolates of *E.coli* and *K.pneumoniae* was found to be 41 and 40% respectively. This is higher than the reported figures of *E.coli* and *K. pneumoniae* in USA (2.2/6.6%), Canada (2.7/6.2%) and India (24.7/38.5) [16,17] and Shahid *et al*., [18] reported the production of ESBL was noticed in 16.9% of isolates, whereas the production of AmpC enzyme was noted in 15.3% of isolates. It must be emphasized here that concurrent occurrence of AmpC and ESBL was noticed in 8.3% of isolates, in which the mechanism could not be inferred by interpretative reading. This is the first report from India regarding the aminoglycoside/cephalosporin resistance mechanisms. The prevalence of AmpC ß-lactamases in this study is significantly high as compared to that found in earlier reported studies from other countries [19,20].

Much higher (58%) prevalence of ESBL producers in urinary isolates of gram negative bacilli was observed in India [16,17,21]. Beta lactamase production has often occurred in parallel with an increase in resistance to aminoglycosides producing highly resistant strains as seen in this study. Shahid *et al*. [18] reported in India the

prevalence of multidrug-resistant bacterial isolates is quite high in our locality. The prevalence of ESBLs by phenotypic NCCLS criteria was found to be 14.3% in *E. coli* isolates and 24.5% in *K. pneumoniae* isolates and the prevalence of AmpC enzymes by phenotypic detection (TDT) was found to be 9.9% in *E. coli* isolates and 31.1% in *K. pneumoniae* isolates. Combinations of amino glycoside modifying enzymes were found responsible for aminoglycosides resistance [18]. Among the aminoglycosides, Amikacin showed best activity (resistance rate 59.5%) as compared to tobramycin (resistance rate 81.31%) and netilmicin (resistance rate 60.39%). And Imipenem showed 100% sensitivity, the another study supported from India the susceptibilities to ESBL producers to imipenem is 100% [15]. The regional variations of resistance to antibiotics may be explained in part by different local antibiotic practices [22,23]. In our study markedly high resistance to cephalosporin and aminoglycosides noted in clinical isolates of *E.coli* and *Klebsiella pneumoniae* is in accordance with other earlier reports [24]. Thus increasing reports of ESBL producing gram-negative infection is of particular concern.

We observed a significant increase in frequency of ESBL-producing *E. coli* in urine samples. ESBL production has been experimental in large proportion of urinary isolates. Differences insusceptibility patterns of organisms and frequency of infection between hospitals and communities make knowledge of local prevalence and resistance data extremely important [15]. This has direct bearing on choice of empiric therapy. Organisms of note in this regard are ESBL producing Gram-negative bacteria. Patients infected with these strains cannot be treated with β-lactam antimicrobial agents and monobactams. Since co-resistance to non-β lactam antibiotics like norfloxacin, co-trimoxazole and gentamicin was observed, amikacin, nitrofurantoin and imipenem are found to be alternative treatment for ESBL producers. Multidrug resistance and ESBL is a common problem in hospital which emphasizes the need for judicious use of antimicrobial agents and their continuous in vitro monitoring.

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