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INCREASING PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASES (ESBLS) PRODUCING E.COLI AND KLEBSIELLA SPP IN OUTPATIENT DEPARTMENTS (OPDS) PATIENTS IN URINARY TRACT INFECTIONS (UTIS) IN TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated group of fast growing enzymes synthesized by the Gram negative bacteria that are causing medicinal crisis. At present, ESBLs has been increasing as a serious pathogen having the property multidrug resistance. So, The present study was undertaken to find out the prevalence of ESBLs positive E. coli and Klebsiella in urinary isolates obtained from various In-patient Departments (IPDs) , Outpatient Departments (OPDs) and Intensive Care units (ICUs).

Methods: Processing of 251 non- repetitive urine samples received during a period of about one year for detection of ESBLs positive Escherichia coli and Klebsiella spp. was done. All suspected isolates of ESBLs producers were confirmed by the Double Disc potentiating discs test, Double disc synergy test and E-Test.

Results: Out of Two fifty one urinary isolates of Escherichia coli and Klebsiella spp. 93 (37.1%) were confirmed as ESBLs producers and 158(62.9%) were non ESBL producers by all the three tests of confirmation. 61 out of 93 (65.6%) were from OPDs and in all IPDs maximum ESBLs producing urinary isolates were obtained from Medicine wards 12/93 (12.9%).

Conclusion: Results indicate that now ESBL producers are increasing in community. So, routine ESBL detection should be made mandatory not only in indoor patient but also in outdoor also. Appropriate use of third generation cephalosporins must be encouraged to reduce the risk of multidrug resistant bacteria and to make an antibiotic policy.

Key words: Extended-spectrum β-lactamases, Gram negative bacteria, E. coli

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infectious disease ranking next to Respiratory tract infection [1] and is an important cause of morbidity and mortality. UTI is also the most common hospital acquired infection approx. 35% of total infection.[2] Bacteria are the major causative organism of UTI and E.coli is the most prevalent-causative bacteria of UTI in community as well as hospital acquired UTIs. [3,4] followed by Klebsiella, Staphylococcus, Proteus & Pseudomonas. These bacteria along with other members of Enterobacteriacae are found to produce an arsenal of enzymes known as extended spectrum β- lactamases. UTI can usually be treated with a short course of antibacterial therapy.[4]

β-lactamases are enzymes which open β-lactam ring (4 carbon atom ring) of penicillin/cephamycins (i.e cefoxitin & cefotetan). β-lactam antibiotics have 4 atom ring known as β-lactam ring. β-lactamases break the β lactam ring open and therefore deactivate the antibacterial activity of drugs. Gradually with

time the property of inactivating the antibiotics spread to other groups of bacteria in Enterobacteriacae and also larger group of antibiotics were inactivated. This property was first documented in 1983 in Germany in K. Pneumoniae [5] & spread quickly to Europe and US and was later known as Extended spectrum β-lactamases.[6,7]

New threat was proposed by Amp C β-lactamases as they confer resistance to cephamycins (7-2 methoxy cephalosporin) and not affected by commercially available β lactamases inhibitors with loss of outer membrane porins provide resistance to carbapenems [8]

ESBLs become clinically important because they destroy cephalosporins given as first line antibiotics in hospital. This may lead to inappropriate treatment and increased mortality. ESBLs producers are multi-resistant to non-lactam antibiotics such as quinolones, tetracyclines, amino glycosides and trimethoprim/cotrimoxazole, narrowing treatment options due to plasmid mediated transfer of resistance and provides therapeutic challenges. [9] Detection of ESBL production by urinary isolates is therefore very important to ensure appropriate antibiotic treatment.

As the prevalence of ESBLs differs significantly both geographically and with different risk factors in patients, knowledge of these variations can help in appropriate and timely antibiotic therapy as well as avoidance of preventable antibiotic use. With reports of high prevalence of ESBL producers and lack of information in India, the present study was carried out to find out the distribution of ESBL producing bacteria isolate from urine in different units of a tertiary care hospital in Jaipur and in community acquired UTIs presenting to this hospital.

MATERIAL AND METHOD

The present study was carried out in the Department of Microbiology, Mahatma Gandhi Medical College and Hospital, Jaipur (Rajasthan).The test group selected was the population of patients from various Out Patient Departments, different wards, and Intensive care units in the hospital regardless of their age, sex, occupation, religion and ethnicity. A Proforma was filled accordingly.

Sample collection

Processing of 251 non- repetitive urine samples received during a period of about one year for detection of ESBLs positive Escherichia coli and Klebsiella spp. was done.

Sample was collected with Universal precautions by prescribed sterile technique. Samples were transported to the laboratory as soon as possible maintaining optimum transportation conditions. Routine microscopy of all urine samples was done and samples with more than /equal to 5 white blood cells /HPF were selected.

Sample culture

All culture Medias were obtained from Hi media Laboratories Mumbai, India. Primary inoculation was done on the Blood agar, MacConkey agar which was incubated 18-24 hrs at 37^0C aerobically.

Escherichia coli and Klebsiella spp. from samples were finally identified by standard techniques based on Colony morphology, Gram's staining, Hanging drop for motility and Biochemical tests as per CLSI guidelines. Antimicrobial susceptibility test using modified Kirby-Bauer disk diffusion method was done of all isolates of Escherichia coli and Klebsiella spp. All the strains identified were tested for ESBLs production as per CLSI guidelines. Following criteria were used in isolates of Escherichia coli and Klebsiella spp. for selection of ESBLs producers:

Screening

Isolates of Escherichia coli and Klebsiella spp. were examined for their susceptibility to 3rd generation cephalosporins Cefotaxime and Ceftazidime antimicrobial discs.

In our isolates of Escherichia coli and Klebsiella spp. the diameter of zone of inhibition was measured for Cefotaxime (30µg) & Ceftazidime (30µg) antimicrobial discs. Diameter of ≤ 22mm for Ceftazidime and/or ≤ 27mm for Cefotaxime was considered as ESBLs suspects as per NCCLS guidelines. [10] All suspected isolates of ESBLs producers of Escherichia coli and Klebsiella were confirmed by the Double Disc potentiating discs test**,** double disc synergy test and few suspected isolates were also be confirmed by E-Test.

Quality control with Standard strains for Escherichia coli ATCC 25922 and Klebsiella spp. 700603 was also done.

RESULTS

The present study was under taken to detect the prevalence and antimicrobial susceptibility of ESBLs positive Escherichia coli and Klebsiella spp. in urinary isolates obtained in Department of Microbiology, Mahatma Gandhi Medical College, and Jaipur.

The samples were obtained from OPDs, IPDs and ICUs of various Department from Mahatma Gandhi Hospital,Jaipur. 251(Two fifty one) urinary isolates of Escherichia coli and Klebsiella spp. were processed for ESBL detection. In primary screening of 251 isolates 97 were found to be resistance to Cefotaxime and/or Ceftazidime antibiotic discs. Those 97 suspects of ESBL producing Escherichia coli and Klebsiella spp.in urinary samples were further tested for confirmation by Double Disc Synergy test and Double Disc Potentiation test .Out of 97 suspects of ESBL producing Escherichia coli and Klebsiella spp.in urinary samples 75 (77.32%) were confirmed by Double Disc Synergy test and 90 (92.78%) were confirmed by Double Disc Potentiation test.25 suspected ESBL producing urinary isolates were tested with E-Test. All except four isolates were tested positive by E-Test. 4(four) isolates were not confirmed positive by any of the three tests hence they were considered non ESBLs producers. Out of 251(Two fifty one) urinary isolates of Escherichia coli and Klebsiella spp. 93 (37.1%) were confirmed as ESBLs producers and 158(62.9%) were non ESBL producers by all the three tests of confirmation.

Out of 93 confirmed ESBLs producers Escherichia coli (89/93) (95.7%) was the predominating organism.52/93 i.e. 56% of ESBLs producing isolates were obtained from female patients. Maximum ESBLs producing isolates were from patients of 20-40 yrs age group followed by 10-20 yrs age group. Maximum ESBLs producing urinary isolates i.e. 61/93 (65.6%) were from Outpatient Departments of various clinical disciplines. Of all In-patient Departments maximum ESBLs producing urinary isolates were obtained from Medicine wards 12/93 (12.9%).

DISCUSSION

ESBLs are an example of the increasing number and diversity of the enzymes that inactivate β-lactam antibiotics. Multi drug resistance and ESBLs enzymes producing E.coli & Klebsiella spp. are major threat to treat urinary tract infections. A dramatic increase in the frequency of ESBL producing E.coli & Klebsiella spp. has been observed in last decade. Frequent and inappropriate use of antibiotics in human led to increasing resistance rate making the treatment of urinary tract infections more complex.

ESBLs has become a major problem worldwide as is confers resistance to the third generation broad spectrum cephalosporins. Although CLSI guidelines exist for detection of E.coli & Klebsiella spp., no such recommendation exists for other ESBLs producing organisms.

Failure of empirical therapy, which is usually, initiated with third generation cephalosporins due to resistance of ESBL producing E.coli & Klebsiella spp. lead to increase in mortality rate in Hospital settings.

Emphasized must be laid on developing rapid screening method for ESBL detection by clinical laboratories so as to report ESBLs producing organisms in appropriate time.

Combination of Antibiotics with their clavulanate salt confirms ESBLs production. Clinical microbiologist play an important role in devising ways and means of rapidly identifying these ESBLs producing organisms and help institutions to initiate appropriate therapy.

Detection become more important by the fact that resistance to one of the 3GC cephalosporins means therapeutic resistance to all cephalosporins up to 3GC even when in vitro sensitivity may be detected otherwise.

In this study observed that female have a higher probability (56%) of predisposition to UTIs. These findings were in accordance with the study by Fennell et al (2008) from Ireland and it was 65.5%.Another study by Babypadmini S et al (2004) from India where they found 56.62 %.[11,12] Therefore our study correlate with various other studies which shows higher percentage of isolates were from female patients. [11,12,13]

Most of the patients in our study were from the age group 20-40yrs (43 %), which shows that UTI is more common in middle age group person in our population compare to Tada Dharmistha G et al 2012 study in which most of the patients were from the age group 10-20 yrs (30%).[13]

The prevalence of ESBLs production in India varies from 5-60%in urinary isolates. In present study the prevalence of ESBLs production was 37% which correlates with MS Kumar et al and S Jalalpour. [17,18] The prevalence of ESBLs producers organisms are on the rising trend as seen in various studies since 2002 to 2012. The prevalence of ESBLs producing E.Coli is 35.45% which correlates with Babypadmini S et al & Shila Jalalpour. Prevalence of Klebsiella spp. is 1.59% which correlates with Joseph Gangoue et al. [12,18]

Our study showed that most of the ESBLs producing isolates were from the Out Patient Departments (Community Acquired).Various studies show that prevalence of Community Acquired UTIs is increasing in recent times. In the beginning ESBLs were in a good number identified to be a hospital based crisis but it is now becoming more common along with community acquired isolates, especially *Esch. coli* (Shila Jalalpour(2011) Iran[18] Parul Agarwal et al 2008 Pune [22]. In the present study *Esch.coli* and *Klebsiella spp.* were found 35.45% and 1.59% respectively.

This was due to illogical and ample use of third generation cephalosporins in both the hospital and community and is alleged to be the main cause of mutations in these enzymes that lead to the appearance of the ESBLs. [23]

Initially ESBL producers were restricted to hospital –acquired infections only, but they have now also been isolated from outpatient departments. Major outbreaks involving ESBL producing strains have also been reported from all over the world.

CONCLUSION

Considering various findings of the present study, it can be concluded that Extended Spectrum beta lactamases are gradually increasing in community in India. ESBL producing organisms have become clinically important in last two decades because of increasing trend in prevalence leading to increase in antimicrobial resistance. Increase antimicrobial resistance result in increase morbidity, mortality and cost of health care.

In order to prevent and control the emergence of antimicrobial resistance in ESBL producing organisms it is of utmost importance to limit the misuse and overuse of antibiotics especially broad spectrum antibiotics.

ESBL producing strains were previously common in Inpatient Departments few years back but nowadays they are commonly isolated

in Outpatient Departments therefore early detection is of clinical importance for effective management of patients.

To conclude, early and sensitive methods to detect ESBL producing strains should be practiced so that appropriate antibiotics may be given to treat the patients and further decrease the spread of ESBL producing microorganisms. It is also utmost important to formulate appropriate hospital antibiotic policies and taking adequate precautionary measures to decrease the spread of ESBL producing microorganisms.

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Table: 1. Percentage of ESBLs isolates in urine samples in different studies

Study group	Total nos. Samples	Nos. of ESBL producers	Percentage
Subha A et al (2002) India [14]	78	4	5.13%
Gangoue JP et al (2005) [15]	124	15	12.1%
Ananthan S et al (2005) India[16]	58	12	20.70%
Ms Kumar et al (2006) India[17]	336	131	39%
Shila Jalalpour(2011) Iran[18]	91	32	35.16%
Present study;Jaipur (2012)	251	93	37%

Table:2 Percentage of ESBL producing strains in different wards

Study groups	OPD	IPD
Khanfar HS et al 2005[19]	51/126 (40%)	75/126 (60%)
Fennell et al 2008[11]	208/464 (45.6%)	256/464 (55.4%)
Meier S et al 2009[20]	79/13 (64.3%)	44/123 (35.8%)
Shakti Rath et al 2011[21]	406/721 (56.31%)	315/721 (43.69%)
Present study Jaipur 2012	61/93 (65.60%)	32/93 (34.40%)

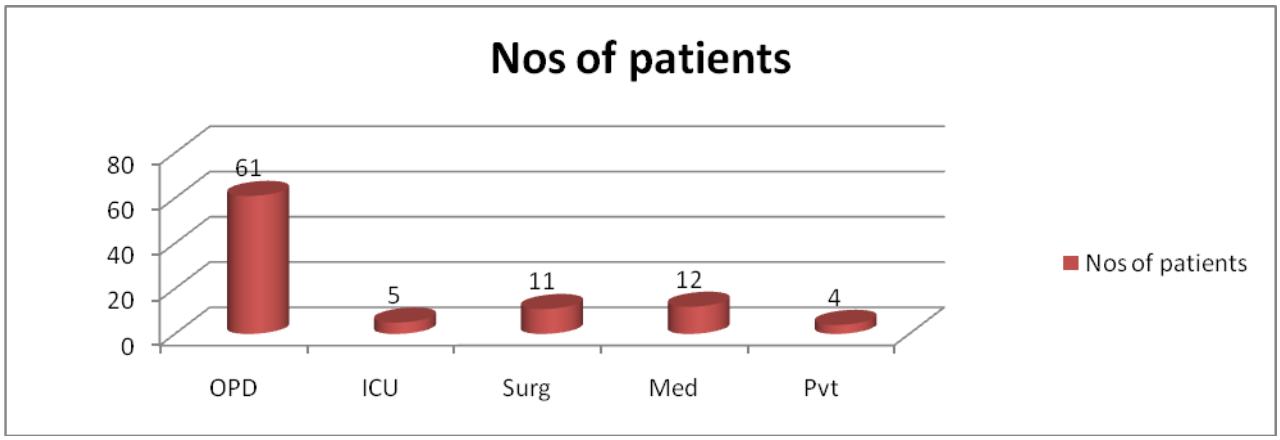

Figure No 1. Showing Ward wise distribution of clinical urinary isolate (ESBLs producers)