1. INTRODUCTION

Urinary tract infections (UTIs) are the most common infectious diseases occurring in nosocomial and community setting. Antibiotic-resistant organisms that cause complicated UTI include Gram positive Staphylococcus aureus, Gram negative organisms particularly those species that produce AmpC enzymes or extended-spectrum β-lactamases (ESBLs) includes, Escherichia coli, Klebsiella, Proteus, Enterobacter, Pseudomonas, and Serratia spp. Urea-splitting organisms such as Proteus spp., Morganella morganii and Providencia stuartii are often found in patients with indwelling devices.

The antimicrobial resistance among uropathogenic bacteria across the globe are emerging. In recent years, spread of plasmid mediated ESBL and various other β-lactamase in gram negative bacteria has risen. This results in an increasing incidence of resistance to third generation of cephalosporin among the urinary pathogens causing a number of therapeutic problems. Penicillins and cephalosporins (the β-lactams) are one of the most valuable groups of antibiotic used in human medicine. The excessive application of these antibiotics is believed to have been a selective force in the emergence of resistance. The emergence and spread of resistance particularly into E. coli (a common cause of gut and urinary infection) is a very serious challenge.

The development of extended spectrum cephalosporin in the early 1980s was regarded as a major addition to our therapeutic armamentarium in the fight against β-lactamase mediated bacterial resistance. The emergence of Escherichia coli and Klebsiella pneumoniae resistant to ceftazidime & other cephalosporins seriously compromised the efficacy of these life saving antibiotics.

ESBL producing organisms are often resistant to several other classes of antibiotics, as the plasmids with the gene encoding ESBLs often carry other resistance determinants. Initially ESBL producing organisms were isolated from nosocomial infections but these organisms are now also being isolated from community.
So, the present study was undertaken to detect the prevalence of various β-lactamase by phenotypic method, which provides a base line survey of drug resistance pattern.

2. MATERIALS AND METHODS

2.1. Specimen collection:
The mid stream urine samples were collected from hospitals and private pathological laboratories. Urine samples were undergone for isolation and identification of bacterial pathogens according to standard microbiological techniques.

2.2. Isolation and identification:
Samples were inoculated on Nutrient and MacConkey's agar for isolation of pathogens. Organisms were grown in pure culture and the samples which have shown significant growth were considered for further study. Isolates were subjected to genus and species level identification by using Hi25-Enterobacteriaceae identification kit based on Bergey’s Manual for Systematic Bacteriology. This kit contains 2 different strips for each isolates. One strip has media for IMViC test as well as others biochemical test and second strip contain various media for testing the results of sugar fermentation. For oxidase test, oxidase reagent disc were used.

2.3. Antibiotic assay:
Antibiotic susceptibility test for β-lactam drugs were done by disc diffusion method. Antibiotic susceptibility was determined by Kirby Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines. Various generation of β-lactam group of drugs were chosen for screening of β-lactam drug resistance as shown in figure-2 for screening the resistance towards β-lactam group of drugs. According to graph 1, out of 152 screened isolates 64 urinary isolates were resistance to 2nd generation and onwards β-lactam resistant.

3. RESULT & DISCUSSION
Total 175 urine samples were collected from hospital and laboratories. Samples were inoculated on Nutrient agar and MacConkey’s agar for isolation of pathogens. 152 out of 175 samples have shown significant growth on media and organisms were identified by standard biochemical tests as shown in figure-1.

As shown in figure-2, various generation of β-lactam drugs including 1st to 4th generation as well as combination β-lactam drugs were placed to screen the resistance towards β-lactam group of drugs. According to graph 1, out of 152 screened isolates 64 urinary isolates were resistance to 2nd generation and onwards β-lactam resistant.
Graph: 1: Screening of urinary isolates to detect the β-lactam resistance.
So 42.10% resistance were observed among this urinary isolates. Out of 64 urinary isolates, 36 isolates were from hospital and 28 were community urinary isolates.

Graph -2: Screening of isolates for evaluation of β lactam resistance
As shown in figure-3, these resistant isolates were undergone for phenotypic detection of various types of β-lactamase according to CLSI guidelines.

Graph: 2: Phenotypic detection of ESBL from hospital isolates.

Graph: 3: Phenotypic detection of ESBL from hospital isolates.
Graph 2 and 3 were showing the ESBL prevalence in nosocomial urinary isolates. Out of 36 screened resistant nosocomial urinary isolates, 29 were showing presence of extended spectrum β-lactamase activity. Some of them have also shown co-resistance with KPC.

ESBLs have been reported from all parts of the world. However, prevalence varies widely even in closely related regions. The true incidence is difficult to determine because of the difficulty in detecting ESBL production & due to inconsistencies in testing & reporting.

In 2007 in Asia pacific region was found to harbour plasmid borne ESBLs 62% and 75% in *E. coli* and *Klebsiella* spp. Respectively. ESBL production rate was 43%, 73.8%, 96% and 70% in *E. coli* and 60% in *Klebsiella* spp. in Pakistan, Iraq, Iran and India respectively in 2009, 2011 and last two were in 2010.10-12
According to graph 4, out of total 28 resistant community isolates 15 were phenotypically detected to be ESBL producing isolates. There were a limited number of studies on prevalence of ESBL showing a high rate in Bangladesh, where E. coli and K. pneumoniae were 43.2% and 39.5% respectively in 2004 and at Rajshahi in Bangladesh it was 57.89% in Klebsiella spp. followed by Proteus spp. 50.0%, E. coli 47.83% and pseudomonas spp. 31.35% in 2010. In France CTX-M-1, CTX-M-3 and CTX-M-14 lactamases from Enterobacteriaceae was isolated in 2002.

Prevalence of KPC in community, according to graph 6 was quite higher than nosocomial isolates. In community urinary isolates the prevalence of KPC was 50% in our study.

Carbapenems are considered the last line of effective therapy available for the treatment of severe infections. Resistance to these agents reduces clinical therapeutic choices and frequently leads to treatment failure.
According to graph-8, the incidence of Amp-C production in community isolates was 7.14%, which is parallel to the resistance found among hospital isolates. Resistance due to plasmid mediated AmpC enzymes is less common than ESBL production in most parts of the world but may be both harder to detect and broader in spectrum. High AmpC production level results in high clinical treatment failures with broad-spectrum cephalosporins. The exact prevalence of AmpC β-lactamases is unknown and this may be due to the absence of simple and reliable detection methods in clinical laboratories.

Phenotypic detection of KPC and Amp-C β-lactamase were shown in figure-4. Hyper production of the K1 enzyme can occur by mutation. The K1 enzyme is predominantly a penicillinase that can also significantly hydrolyze aztreonam, cefuroxime and ceftriaxone and has weak activity against cefotaxime or ceftazidime.

Very few isolates were found to be K1 β-lactamase producing strains. As shown in graph-9, the K1- β lactamase production among hospital urinary isolates were 8.33 %, while according to graph-10, community isolates were found to be only 3.57% resistant.

4. CONCLUSION
Increasing levels of resistance are being found among common community-acquired urinary pathogens, and nosocomial pathogens. Antibiotic regimes which traditionally have been effective are now failing at increasing rates. Over the past few years the incidences of ESBL producing strains were not only restricted to hospitals but they have also been isolated from infections in outpatients. Proper use of antibiotics is very important for various reasons. It reduces unnecessary expenses, reduces development of resistance to useful and life saving antibiotics, and minimizes many side effects. Knowledge of resistance pattern of bacterial strains in a geographical area will help to guide the appropriate and judicious antibiotic use. The correct management of such infections is extremely important for the future, particularly in term of reducing the incidence of new antibiotic resistance patterns.

ACKNOWLEDGEMENT:
We are thankful to the Management, Virani Science College, Rajkot for providing the infrastructure facility and instruments available in the college.

REFERENCES


