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Phenotypic Detection Of Amp C B-Lactamases In Urine Isolates Of E. Coli& Klebsiella Species At A Tertiary Care Centre.

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Abstract:
Background:*In recent years, prevalence of infection with multidrug resistant Enterobacteriaceae has steadily increased.Plasmid mediated Amp C β-lactamases(PMABLs) represent a new threat, as they confer resistance to Cefamycins& thus with loss of outer membrane porin channels, can confer resistance to Carbapenems. These are evolved by the movement of chromosomal genes onto plasmids, and are seen in E. coli, Klebsiellasps, Salmonella sps, Citrobacterfreundii, &Enterobacteraerogenes. Detection of Amp C production is important, as isolates appear susceptible to Cephalosporins but lead to treatment failure.*
Materials & Methods:*1436 urine samples from suspected cases of UTI were collected & processed according to standard microbiological procedures. Isolates of E.coli and Klebsiella species were screened for Cefoxitin susceptibility and those which were resistant to Cefoxitin were further subjected to phenotypic confirmatory tests by Cefoxitin-Cloxacillin double disk synergy test[CC-DDS Test] and AmpC E-Test.*
Results: *Out of 1436 urine samples processed during the study period, 1252 were sterile while 184 were culture positive. Among these, E.coli isolates were 22.8%(42), Klebsiellasps were 40.7%(75) and 36.5%(67) were other isolates like Citrobacter species, Staphylococcus auresu, Proteus species, CONS, Pseudomonas species and Enterobacter species. 42% of E.coli isolates and 44% of Klebsiella isolates tested positive for Cefoxitin screening test, out of which, 35.7% of E.coli isolates and 37.3% of Klebsiellasps showed AmpC production by phenotypic methods. [CC-DDS Test and Amp C E-Test].*
Conclusion: *CC-DDS test showed the same results compared to E-Test. It is also cost effective & easy to perform. Hence it can routinely be used in labs for AmpC detection.*
Key words: *Amp C, E. coli, Klebsiellasps, multidrug resistance,UTI.*

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I. Introduction:

Amp C β-lactamase production is one of the mechanisms of resistance to a wide variety of β-lactam antibiotics, in Gram Negative bacteria like Escherichia coli, Klebsiellapneumoniae, Proteus mirabilis, Citrobacterfreundii, Enterobacteraerogenes and Salmonella sps. Amp C β-lactamases are included in class C of Ambler's classification of βlactamases. The organisms producing these enzymes are resistant to all Penicillins, 1st,2nd& 3rd gen Cephalosporins and also to cephamycins(cefotetan and cefoxitin) but remain sensitive to Carbapenems. There are 2 types of Amp C β-lactamases: Chromosomal inducible and non-inducibleplamid mediated Amp C β-lactamases(PMABLs). PMABLs have evolved by the movement of chromosomal genes on to plasmids and are seen in many Gram negative bacilli. This resistance is seen in E. coli&Klebsiellasps around the world, causing nosocomial outbreaks. Detection of Amp C production is important, as isolates appear susceptible to Cephalosporins but lead to treatment failure.

Phenotypic screening & confirmation tests are inexpensive but highly sensitive & specific. Therefore, this study was undertaken to detect PMABLs in E. coli&Klebsiellasps, which are the most common uropathogens.

II. Materials & Methods:

A cross-sectional study was conducted in the Dept of Microbiology, at Osmania General Hospital, Hyderabad, from October 2019 – December 2019. Institutional Ethics Committee approval was obtained prior to the study. Both male & female patients of >16 years' age group, who presented to the OPD with symptoms of UTI, and the in-patients who had symptoms of UTI, were included in the study. Samples other than urine, as

well as isolates other than E. coli&Klebsiellasps, were excluded from the study. Consent was obtained from those who were willing to participate in the study.

Urine samples from suspected cases of UTI were collected & processed according to standard microbiological procedures. AST was done by Kirby Bauer disk diffusion method againstCefoxitin(CX), Ampicillin(AMP),Nitrofurantoin(NIT), Ciprofloxacin(CIP), Gentamicin(GEN), Pieracillin-Tazobactam(PIT), Cefotaxime(CTX), Imipenem(IPM)&Cefipime(CPM).E. coli and Klebsiella species were isolated & screened for Cefoxitin susceptibility. The isolates which were resistant to Cefoxitin, i.e zone diameter <14mm were further subjected to phenotypic confirmatory tests by Cefoxitin-Cloxacillin double disk synergy test(CC-DDS) and AmpC E-Test.

1)Cefoxitin-Cloxacillin Double Disk Synergy Test (CC-DDS):

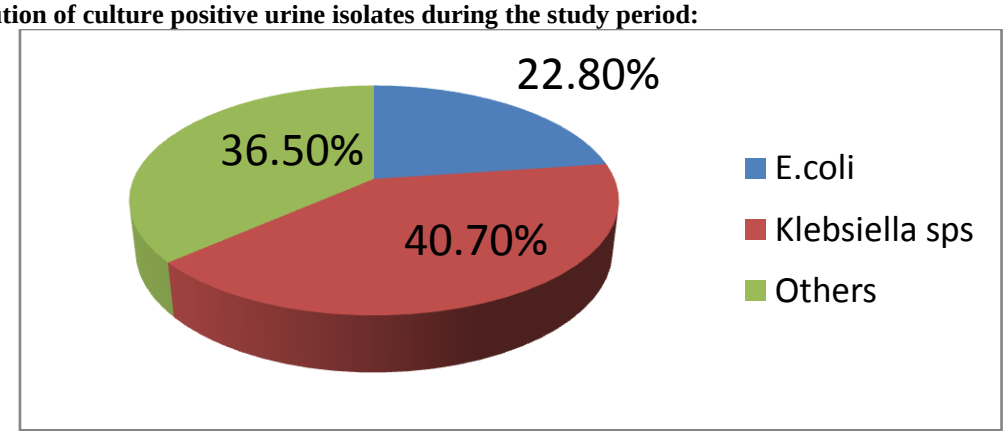
- Isolates tested positive for Cx screening test were lawn cultured on MHA plates.
- Cx disk (30µg) &Cx + Cloxacillin (30/200µg) disks were applied.
- Inoculated plates were incubated overnight @37°C.
- An increase in zone diameter of ≥4mm with CCX disk than CX disk alone was considered as Amp C producer.

2)Amp C E-strip test:

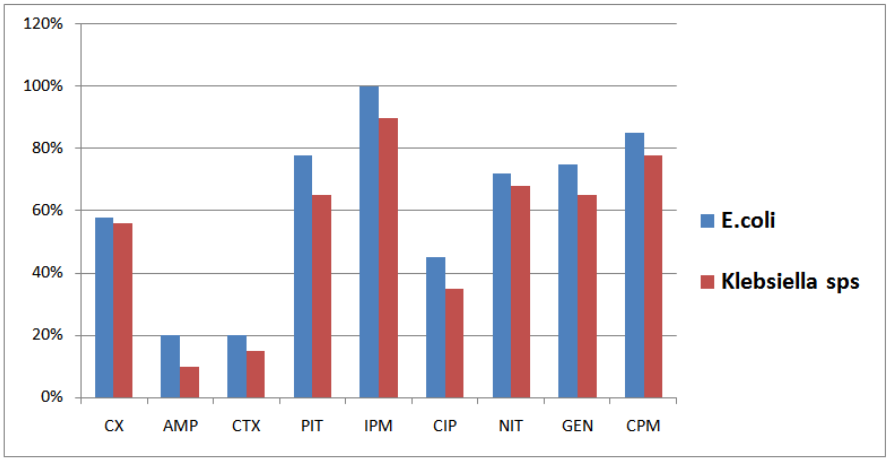
- The strip consists of Cefotetan(CTN) and Cefotetan + Cloxacillin (CNI) in a gradient concentration on both the halves.
- Isolates were lawn cultured on MHA plates and E-strips applied.
- Inoculated plates were incubated overnight @ 37°C.
- Ratio of MIC values of CN/CNI of ≥8 were considered as Amp C producers.

III. Results:

Distribution of culture positive urine isolates during the study period:

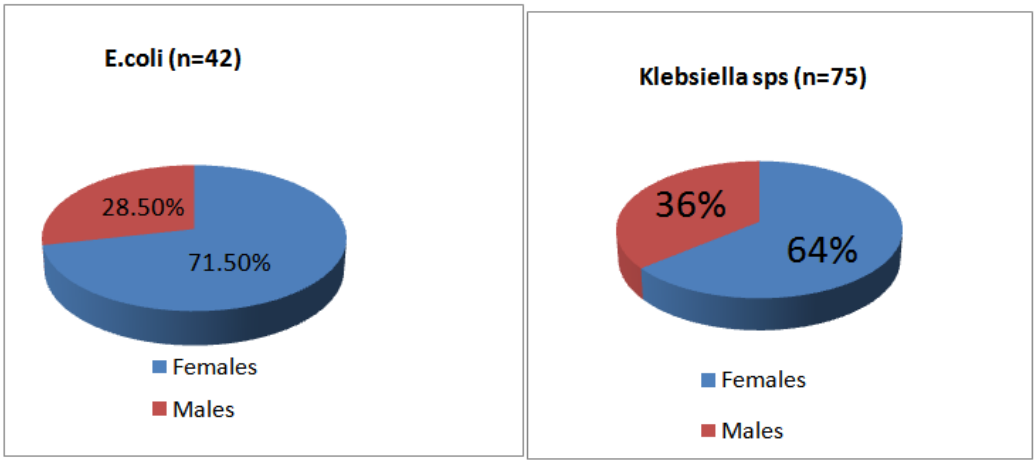


Antibiotic sensitivity pattern of E. coli&Klebsiella isolates:



Both E. coli&Klebsiellasps showed maximum sensitivity to Imipenem(Carbapenems) but showed resistance to Ampicillin and 3rd generation cephalosporinsi.eCefotaxime. Variable amount of resistance was seen against Nitrofurantoin, Ciprofloxacin and Gentamycin.

Gender-wise distribution of E.coli&Klebsiella isolates:



Amp C Detection Methods used in the study:

Isolates	CX screening test	CC-DDS Test	Amp C E-test
E.coli (n=42)	18(42%)	14(33.3%)	15(35.7%)
Klebsiellasps (n=75)	33(44%)	26(34.6%)	28(37.3%)

IV. Discussion:

STUDY DONE BY:	% of PMABL producing E.coli	% of PMABL producing Klebsiellasps
Paul R.Ingram et.al (June 2011)	39%	14%
Vera Meyer et.al (Feb 2011)	2.30%	0.90%
Thean Yen Tan et.al (June 2008)	40.80%	57.80%
Present Study	35.70%	37.30%

V. Conclusion:

Lack of consensus regarding optimal methods for Amp C detection is a major barrier to understand their clinical significance. Therefore, a mechanism based approach i.e. a Screening test followed by a Confirmatory test will reduce erroneous cephalosporin susceptibility testing.

- Sensitivity of screening test varies from 77-99% whereas specificity ranges from 89-95%.
- Sensitivity of phenotypic confirmatory tests: 90-99%.
- Specificity of phenotypic confirmatory tests: 95-100%.

These tests can be routinely employed in the labs as they have high sensitivity & specificity and also inexpensive.

Dissemination of PMABL producing strains within the hospitals is an important public health issue.

Hence, detection of Amp C producing strains helps in initiating appropriate antibiotic therapy & preventing nosocomial outbreaks.

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