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PREVALENCE of AmpC β-LACTAMASE IN PLASMID RESISTANT GENE FROM UTI CLINICAL ISOLATES BY MOLECULAR TECHNIQUES

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Keywords:

AmpC β-lactamase, Extended spectrum β-lactamase, IMVIC, DMS, Modified-disc method, Etbr, PCR

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ABSTRACT: Objective: To detect the prevalence of Plasmid resistant AmpC β-lactamases gene in clinical isolates of gram negative organisms from UTI patients which produce resistance against multiple antibiotics. The gene coding for AmpC β-lactamases is also present in *E. coli* & *Klebsiella* species was not expressed because due to lack of promoter region, but the transfer of chromosomal genes to plasmids allows the expression of AmpC β-lactamases that hydrolyze the β-lactam ring, which has greater impact on resistance against multi-drug antibiotics, is a significant problem around the world. **Methods:** Among 20 non-repetitive clinical isolate of each *Escherichia coli* and *Klebsiella pneumoniae*, examined for identification and characterization of urine cultures based on morphological, biochemical tests, antibiotic resistant pattern, modified-disc method and detection of AmpC gene by plasmid identification by agarose gel electrophoresis and amplification of AmpC gene by PCR techniques. **Results:** The study detects the prevalence of AmpC gene primarily by modified disc inducer method as well as conformational molecular analysis by PCR amplification techniques. AmpC prevalence was observed in both strains from the UTI clinical isolates. The prevalence of AmpC resistance may differ due to the geographical variations was observed in different strains of gram negative organisms. **Conclusion:** The detection of AmpC resistance mechanism in plasmid DNA is an important factor to improve the clinical management of infection against higher antibiotics in relation with antibiotic resistance and cost of antibiotics which could help in therapeutics and UTI control process.

INTRODUCTION: Urinary tract infection is one of the most common infections observed in hospital practices, caused by uro-pathogens within any of the structures that comprise the Urinary Tract Infection. The incidence of nosocomial UTIs has been increasing resistance against multiple higher antibiotics because of mutated pathogens and its treatment has become more complicated in extensive manner.

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All individuals are susceptible to urinary tract infection differs respective with age, sexual activity, anatomical structures and geographical nature. Resistance to expanded-spectrum Cephalosporin's may develop through the expression of chromosomally encoded class C β-lactamases, also known as AmpC β-lactamases. AmpC β-lactamases enzymes encoded by both chromosomal and plasmid genes are also evolving to hydrolyze broad-spectrum cephalosporin's more effectively and efficiently.

Several phenotypic screening methods used in clinical microbiology for the detection of PABLs in clinical isolates were under practices. Factors, such as multiple beta-lactamase production, transferable multiple-resistance genes, alterations in outer-

membranes porins and antibiotics afflux may also contribute to a phenotype resistance. Inhibitor-based screening methods would improve detection of this emerging resistance phenotype. The inhibitor based confirmatory method, paved a way to detect AmpC β-lactamases enzymes, the most common predominant mechanism of resistance in gram negative bacteria after the ESBL production. AmpC and ESBL enzymes are present together can mask the phenotype of each other.

Hence, plasmid mediated AmpC β-lactamases gene producers were resistance to cefoxitin drugs therefore, cefoxitine was considered as a better plasmid mediated AmpC β-lactamases screening indicator than cefotetan. AmpC Producers were resistant to cefoxitin or sensitivity to cefoxitin could be used to rule out the possibility of PABLs producers. The use of phenyl boronic acid in combination with cefoxitin as one type of phenotypic screening method shows better potential in detection of AmpC gene.

MATERIALS AND METHODS:

Isolation and Identification of *Escherichia coli* and *Klebsiella pneumoniae* Isolates from Urine Cultures: A total number of 20 non-repetitive clinical isolates of *Escherichia coli* & *Klebsiella pneumoniae* of each was collected during 3 month period in Muller-Hinton agar slants from UTI patients from a hospital in Coimbatore and stored in refrigerator at 4^0C.

Lactose fermenting colonies on EMB agar and Mac-Conkey agar with significant bacteriuria were processed and identified as *Escherichia coli* and *Klebsiella pneumoniae* by performing standard biochemical tests-IMVIC, H_2S production and carbohydrate fermentation (Glucose, Lactose and Sucrose) as per standard protocol.

Morphological Identification:

Identification and Characterization of *E. coli* and *K. pneumoniae* Isolates by Gram staining: A smear was prepared using a clean, dry, grease-free glass slide by an inoculating loop. The smear was allowed to air-dry and then heat fixed. The smear was flooded with crystal violet and allowed to stand for 1 min and then washed with tap water. The slide was then flooded with Gram's iodine and allowed to stand for a minute. The smear was then washed with tap water. De-colorisation was done using 95% alcohol and the slide was washed again with tap water. Counterstained with safranin for 45 seconds and washed again with tap water. Finally, the slide was blotted dry with bibulous paper and examined under oil immersion.

Motility by using Hanging-Drop method: A loop of culture was then placed in the center of a cover slip; a thin layer of petroleum jelly was applied along the edges of a cover slip. A clean glass depression slide was placed over the cover slip with the concave surface facing down, to avoid the drop of culture touching over the depression slide. The slide gently pressed to fix firmly with the cover slip and inverted so that the drop continued to adhere to the inner surface of the cover slip. The slide was examined under the microscope by focusing the edge of the drop culture under the low power objective and finally reducing the light source using condenser and observing under high power objective.

Biochemical Tests: [IMViC TEST]

i. Indole Test: Following sterile technique, each organism was inoculated into appropriately labeled SIM agar deep tubes by stab inoculation. The inoculated tubes were incubated for 24 to 48 h at 37°C. The incubated test tubes were gently removed and 10 drops of Kovac's reagent was added. Colour obtained in the test tube was examined and recorded. One tube served as a control.

ii. Methyl Red Test: Following sterile technique, each organism was inoculated into appropriately labeled MRVP broth tubes. The inoculated tubes were incubated for 24 to 48 h at 37°C. The incubated test tubes were gently removed and 5 drops of methyl red indicator was added. Colour obtained in the test tube was examined and recorded. One tube served as a control

iii. Voges-Proskauer test: Following sterile technique, each organism was inoculated into appropriately labeled MRVP broth tubes. The inoculated tubes were incubated for 24 to 48 h at 37°C. The incubated test tubes were gently removed and 10 drops of Barritt's reagent A was added and the tubes were shaken well.

Immediately, 10 drops of Barritt's reagent B was added and shaken well. The culture tubes were slightly shaken every 2-3 min and the colour obtained in the test tube was examined and recorded. One tube served as a control.

iv. Citrate Utilization Test: Following sterile technique, each organism was inoculated into appropriately labeled Simmon's citrate agar slants tube. The inoculated tubes were incubated for 24 to 48 h at 37°C. The agar slant was examined for change in the colour of the medium from Green to Prussian blue. One tube served as a control.

v. Hydrogen Sulphide Test: Following sterile technique, each organism was inoculated into appropriately labeled SIM agar deep tubes by stab inoculation. The inoculated tubes were incubated for 24 to 48 h at 37°C. The tubes were examined for the presence or absence of black colouration along the line of stab inoculation and the result was recorded. One tube served as a control

vi. Carbohydrate Fermentation: Following sterile technique, each organism was inoculated into appropriately labelled fermentation broth tubes containing phenol red, (lactose or sucrose or glucose) broths along with Durham tubes. The inoculated tubes were incubated for 24 to 48 h at 37°C. All carbohydrate broth cultures were examined for change in colour and presence or absence of a gas bubble was observed and recorded. One tube served as a control.

Preparation of 0.5 Mc Farland Standards: Preparation of 0.5Mc Farlands standards by mixing 0.5 ml of 0.048 M $BaCl_2$ (1.175% w/v barium chloride dehydrate) with 99.5 ml of 0.35 N H_2SO_4 (1% v/v). This produced an inoculating suspension of approximately 10^8 CFU per ml, then diluted in fresh broth to final an inoculum of approximately 10^5 CFU/ml.

The standardized bacterial cultures were preceded for the other tests, which were done in triplicates. The clinical isolates pre-cultured in Muller Hinton broth and incubated at 37 ºC for 24 h. The inoculum was standardized by matching the turbidity of the clinical cultures visually to 0.5 Mc Farlands standards.

Antibiotic Sensitivity Testing to Select the Multi-Drug Resistance Strain (MDR's): Antibiotic sensitivity testing to select the multi-drug resistance strain (MDR's) of the 20 consecutive non-repetitive clinical isolates of *Escherichia coli* & *Klebsiella pneumoniae* of each were carried out on Mueller-Hinton agar plates by Kirby-Bauer disc diffusion technique.

Mueller-Hinton agar plates were prepared and then the characterized UTI clinical isolates were inoculated on the plates by using a sterile cotton swab. The different antibiotic standard disc were then placed on the petriplate and kept in a refrigerator for 15 min for diffusion. The plates were finally incubated for 24 h at 37 °C and zone of inhibition was observed and recorded.

Screening Test for AmpC β- lactamase by Phenyl Boronic-Cefoxitin Disc Method of MDR Strain:

Preparation of Cefoxitin-Phenyl Boronic Acid Disc: The stock solution of 120 mg phenyl boronic acid was dissolved in 3ml of dimethyl suphoxide and 3 ml of sterile distilled water was added to this solution. Pipette out 20 µl of Phenyl boronic acid (400 µg) from the prepared stock solution and dispensed onto cefoxitin 30 µg standard disc. Then the disc was allowed to dry for 20-30 min.

Methods of AmpC Detection: Overnight, cultures of clinical isolates were swabbed with sterile cotton swab on Muller Hinton agar plates. Disc susceptibility testing was performed by antibiotic standard disc containing cefoxitin 30 µg and a similar standard disc containing cefoxitin 30 µg supplemented with 400 µg of phenyl boronic acid were placed on the swabbed agar plates at a distance of 30 mm and kept aside for 10-15 min for diffusion. Incubate the plates at 37 ºC for 24 h, zone of inhibition observed and measured with antibiotic zone scale.

Genetic Evaluation of Selected MDR Clinical Isolates:

Isolation of Plasmid DNA: Plasmid DNA isolation from the bacterial cells (clinical strains) was done by alkaline lysis method. The selected resistance strains transferred in aseptic hood from Muller Hinton agar slants to Luria Bertani agar slants and stored at 4 ºC in refrigerator to preserve

the master culture. Simultaneously, they are inoculated in 10 ml LB broth with antibiotic and grown over night at 37 ºC.

After 24 h of incubation, 2 ml of saturated bacterial culture was pipetted in 4 ml centrifuge tubes, spun at 10,000 rpm for 1 min and the supernatant solution was discarded. Again, the process was repeated once to recover more number of the cell pellets. To the cell pellets add 0.2 ml of ice-cold solution I (Glucose, Tris HCl (pH 8.0) & EDTA (pH 8.0) and re-suspend the cells thoroughly. Again, add 0.4 ml solution II (1% SDS & 0.2 N NaOH) to this and invert gently 5 times, keep the centrifuge tubes aside at room temperature for 5 minutes. Then add 0.3ml of ice-cold solution III (Potassium acetate & Glacial acetic acid) and again inverting the tubes gently for 5 times, cool the tubes for 10 min on the ice-cold gel pack.

Centrifuge the tubes for 5 min. Transfer the supernatant to fresh 4 ml centrifuge tubes using clean micropipette tips. Leave of the white precipitates while transferring the supernatant solution. Fill the remaining volume of the centrifuge tubes with isopropanol and kept it at room temperature for 2 min. Again centrifuge the tube once for 5 min and decant the supernatant solution from the centrifuge tube without dumping the milky white pellets. Finally, add 1 ml of ice-cold 70% ethanol, invert the centrifuge tube several times and spin the tubes for 1 min. Taking care, decant the supernatant without dumping out the pellets.

Air-dry the pellets in the centrifuge tubes until the ethanol evaporates. Add 50 µl of TE buffer (Tris – EDTA) to all the isolated tubes. Centrifuge the tube to re-suspend the white pellets completely in the buffer solution. The isolated plasmid DNA obtained from the bacterial cells was kept at room temperature for 5 minutes before storing at -20 °C.

Weight 0.45 grams 1.8% agarose in a clean 50 ml beaker, to this add 0.5 ml 50 X TAE buffer and 24.5 ml distilled water and heat it till the agar melts to form a clear solution. Then add a pinch of ethidium bromide to the clear solution and solidify it in the gel tray with required combs (comb type 4, 5 & 8).

From the 50 µl of the isolated plasmid DNA, 10µl was loaded to the agarose well with 5 µl of 6X gel loading buffer and run by electrophoresis unit at 50-100 volts for 2 - 3 h till the tracking dye reached $3/4^{th}$ length of the gel, the DNA fragments visualized under UV trans illuminator and the photo image on the gel was recorded.

AmpC Determination by PCR Amplification Technique:

AmpC primer sequence

F -5'-AATGGGTTTTCTACGGTCTG-3'

R-5'-GGGCAGCAAATGTGGAGCAA-3'

PCR reaction was performed in PCR tubes, 50 µl as final volume. Thaw the primers, nuclease free water and isolated plasmid DNA kept aside on ice-cold gel pack for the PCR reactions.

In a fresh PCR tubes add 25 µl of master mix and then add 2 µl of the forward primer and 2 µl of reverse primer. To this mix, add 2 µl of plasmid DNA and 19 µl of nuclease free water as final volume to 50 µl. Prepared 50 µl PCR sample was then centrifuged with mini-spin for proper mixing and reaction were carried out by PCR amplification technique for the following thermo cycle processes,

Event 1: (Cycle 2 Steps 1 repeats) - Initial denaturation step:

Step 1:

Temperature- 94 ºC

Time-1 minutes 20 sec

Step 2:

Temperature- 94 ºC

Time-1 minutes 20 sec

Event 2: (Cycle 3 Steps-25 repeats)- Amplification step:

Step-1(Denaturation)

Temperature- 94 ºC

Time-45 sec

Step 2: (Annealing)

Temperature-51 ºC

Time-45 sec

Step 3: (Extension)

Temperature-72 ºC

Time-60 sec

Event 3: (Cycle 4 Step-1 repeats) - Final extension:

Step 1:
Temperature-72 ºC
Time-1 minutes 20 sec

Step 2:
Temperature-72 ºC
Time-1 minutes 20 sec

Step 3:
Temperature-72 ºC
Time-1 minutes 20 sec

Step 4
Temperature-72 ºC
Time-60 sec

Event 4: (hold)
Temperature- 4 ºC
Hold in time for 60 sec

After PCR amplification, 15µl of PCR amplified product with 5 µl of 6 X gel loading buffer was loaded to the 2% agarose well, stained with ethidium bromide. The amplified products are performed using electrophoresis unit at 50-100 volts for 2 - 3 h until the tracking dye reaches 3/4th length of gel. Interpretation of amplified product was compared with DNA ladder on the gel visualized under UV trans illuminator (Alpha digidoc) and photo images are recorded. The remaining PCR amplified products was stored at -20 ºC until it requires for sequencing.

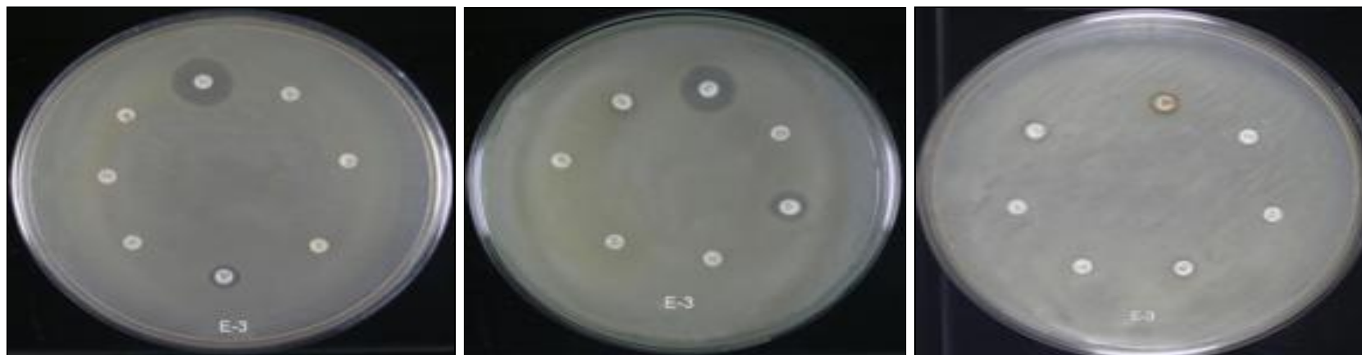
RESULTS:

TABLE 1: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF CLINICAL SAMPLES

S. no.	Labelled culture	Gram nature (rod)	Motility	Biochemical tests					Carbohydrates Fermentation		
				Indole	Methyl red	Voges-Proskauererr	Citrate	H_2S	Glucose	Lactose	Sucrose
1-20	*Escherichia coli*	-	+	+	+	-	-	-	AG	AG	A±
1-20	*Klebsiella pneumoniae*	-	-	-	±	±	+	-	AG	AG	AG

(+) indicates for positive reaction, (-) indicates for negative reaction.
AG-acid and gas, ± -Variable reaction

Out of 20 *Escherichia coli* isolates tested, 15 shows positive results for all tests and 5 shows negative results for the same. Out of 20 *Klebsiella pneumoniae* isolates tested, 9 shows positive results for all tests and 11 shows negative results for the same. The clinical isolates which showed positive results for all the tests given in the data sheet was confirmed and recorded. Such conformational isolates were preceded for further evaluation.



***Escherichia coli* strain**

FIG. 1: ANTIBIOTIC DISC SUSCEPTIBILITY TESTING OF CLINICAL ISOLATES

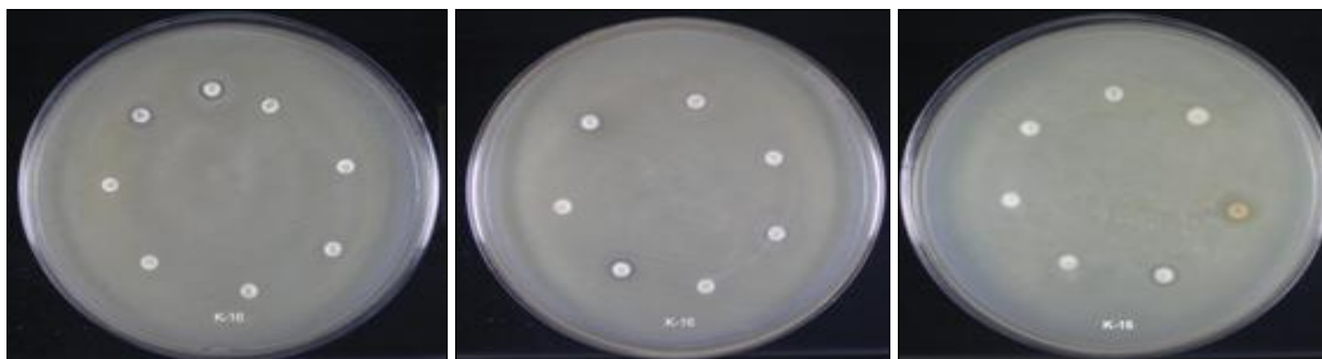
Out of 15 *Escherichia coli* isolates tested for antibiotics sensitivity, 6 showed resistance to a minimum of 11 antibiotics and a maximum of 16 antibiotic, while 9 showed sensitivity to a minimum 7 and maximum of 11 antibiotics tested for the same.

TABLE 2: RESISTANCE AND SENSITIVE PATTERN OF 6 *ESCHERICHIA COLI* STRAINS

S. no.	Labelled strains	Antibiotic resistance pattern	Antibiotic shows resistance to all strains	Antibiotic shows minimum resistance
1	E-3	A,AM,P,CF,OF,CFM, CFP,CQ,CN,CH,CZ, CTX,CTN,CEP,CTR,CU		
2	E-4	A,AM,P,CF,OF,CU, CEP,CTR,CZ,CFM, CQ,CFP,CH,CN	 A P	CQ (5) CN (5) CH (4)
3	E-6	A,AM,P,CF,OF,CEP, CTR,CTX,CU,CFP,CFM	CF OF	CZ (5) CFP (5)
4	E-9	A,AM,P,CF,OF,CZ, CFM,CQ,CN,CFP, CH,CEP,CU,CTR	CU AM CEP	CTX (2) CTN (1) CTR (4)
5	E-13	A,AM,P,CF,OF,CN,CFP, CZ,CFM,CQ,CH,CU,CEP		CFM (5)
6	E-17	A,AM,P,CF,OF,CH,CQ, CN,CZ,CFP,CU,CEP		

TABLE 3: PERCENT RESISTANCE AND SENSITIVE PATTERN OF 6 *ESCHERICHIA COLI* STRAINS

S. no.	Labelled strains	Total no. of antibiotics disc used (22)	
		Resistance in percentage	Sensitive in percentage
1	E-3	16 (72.72%)	6 (27.27%)
2	E-4	14 (63.63%)	8 (36.36%)
3	E-6	11 (50%)	11 (50%)
4	E-9	14 (63.63%)	8 (36.36%)
5	E-13	13 (59.09%)	9 (40.90%)
6	E-17	12 (54.54%)	10 (45.45%)



***Klebsiella pneumoniae* strain**

FIG. 2: ANTIBIOTIC DISC SUSCEPTIBILITY TESTING OF CLINICAL ISOLATES

Of the 9 *Klebsiella pneumoniae* isolates tested for sensitivity, 3 showed resistance of minimum 13 and maximum of 15 antibiotics, while 6 shows sensitive to minimum 5 and maximum to 9 antibiotics tested for the same.

TABLE 4: RESISTANCE AND SENSITIVE PATTERN OF 3 *KLEBSIELLA PNEUMONIAE* STRAINS

S. no.	Labelled Strains	Antibiotic resistance pattern	Antibiotic shows resistance to all strains	Antibiotic shows minimum resistance
1	K-3	A,AM,P,CF,OF,CEP,CU,CTN, CN,CQ,CFP,CH,CZ	 A,P,CF,	 CFM (2)
2	K-11	A,AM,P,CF,OF,CEP,CTR,CU,CH, CZ,CFP,CN,CFM,CQ	OF,CU,CQ, CZ,CN,CH,	CTX (2) CTN (1)
3	K-16	A,AM,P,CF,OF,CU,CN,CH,CEP, CZ,CFP,CQ,CFM,CEP,CTR	AM,CFP,CEP	CTR (1)

TABLE 5: PERCENT RESISTANCE AND SENSITIVE PATTERN OF 3 *KLEBSIELLA PNEUMONIAE* STRAINS

S. no.	Labelled Strains	Total no. of Antibiotics disc used (22)	
		Resistance in percentage	Sensitive in percentage
1	K-3	13 (59.09%)	9 (40.90%)
2	K-11	14 (63.63%)	8 (36.36%)
3	K-16	15 (68.18%)	7 (31.81%)

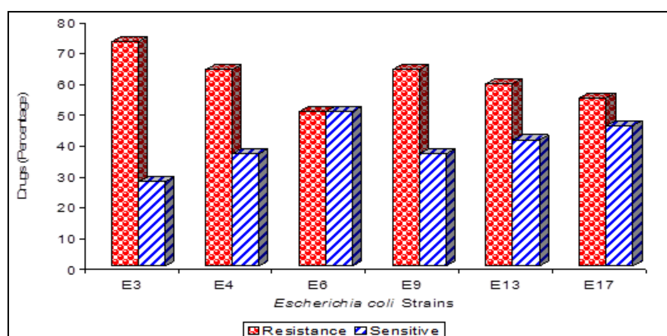


FIG. 3: PERCENT RESISTANCE AND SENSITIVE PATTERN OF 6 *ESCHERICHIA COLI* STRAINS IN BAR DIAGRAM

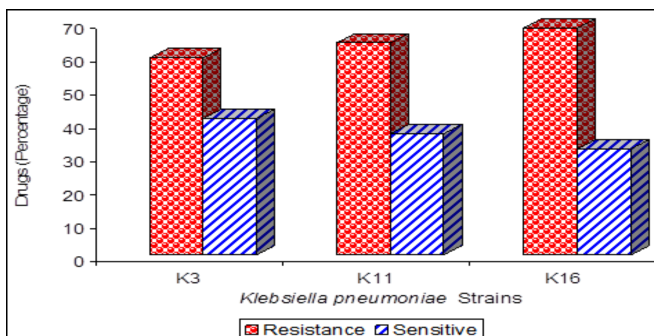
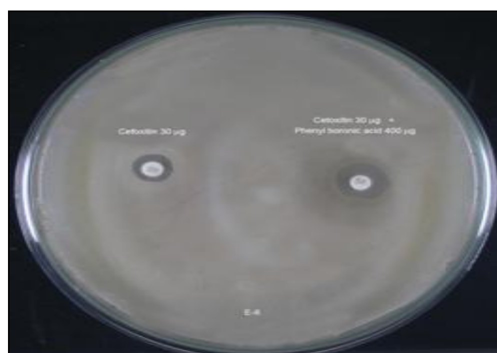
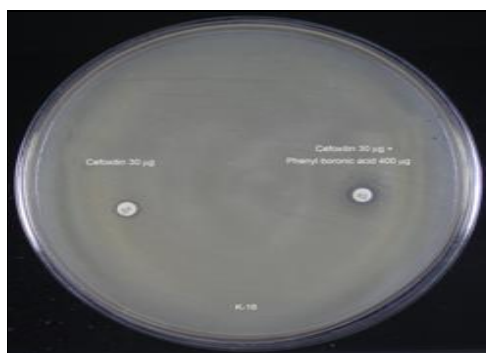


FIG. 4: PERCENT RESISTANCE AND SENSITIVE PATTERN OF 3 *KLEBSIELLA PNEUMONIAE* STRAINS IN BAR DIAGRAM



***Escherichia coli* strain**



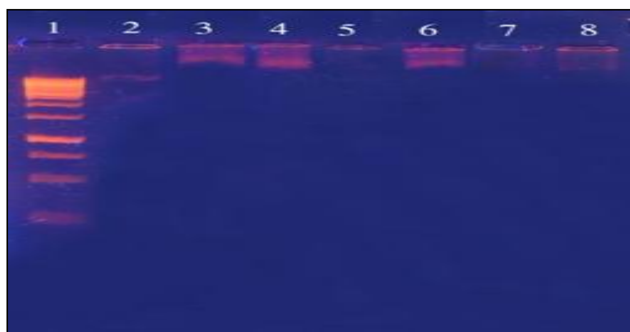
***Klebsiella pneumoniae* strain**

FIG. 5: SCREENING TEST FOR AmpC β- LACTAMASE USING MODIFIED DISC-METHOD WITH CEFOXITIN-PHENYL BORONIC ACID

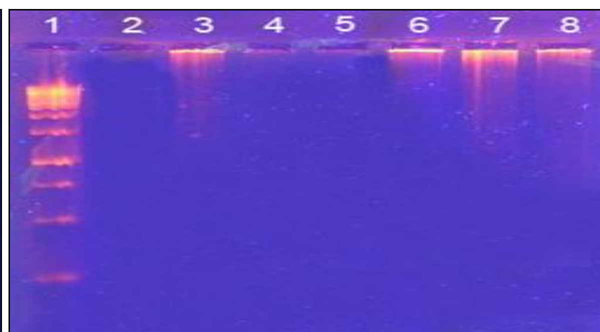
Out of 9 resistant strains, 6 *Escherichia coli* strains and 3 *Klebsiella pneumoniae* shows an increase of ≥ 5mm in the zone of inhibition for the cefoxitin-phenyl boronic disc as compared with the cefoxitin disc, which was interpreted as a positive test for the AmpC producing strain based on phenotypic interpretation. The positive strain was carried out for the detection for resistant plasmid by agarose gel electrophoresis.

Genetic Evaluation of Selected Clinical Isolates:

Isolation of Plasmid DNA



Escherichia coli



Klebsiella pneumonia

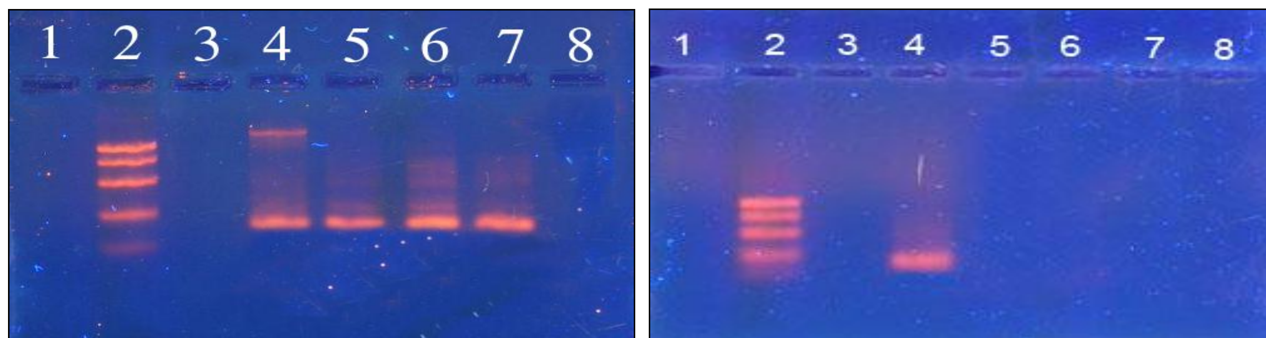
FIG. 6: GENETIC EVALUATION OF SELECTED CLINICAL ISOLATES

Lane 1: DNA LADDER
Lane 2: E-4
Lane 3: E-9
Lane 4: E-3
Lane 5: E-4
Lane 6: E-13
Lane 7: E-6
Lane 8: E-17

Lane 1: DNA LADDER
Lane 3: K-3
Lane 6: K-11
Lane 7: K-3
Lane 8: K-16

The selected 6 *Escherichia coli* and 3 *Klebsiella pneumoniae* isolates were evaluated out for plasmid DNA isolation. The isolated plasmid DNA from clinical isolates was identified and processed for PCR amplification technique.

AmpC Determination by PCR Techniques:



Escherichia coli ***Klebsiella pneumoniae***

FIG. 7: AmpC DETERMINATION BY PCR TECHNIQUES

Lane 2: DNA LADDER
Lane 4: E-4
Lane 5: E-3
Lane 6: E-13
Lane 7: E-9

Lane 2: DNA LADDER
Lane 4: K-16

Of total 9 AmpC positive strains based on phenotypic evidence, 5 strains showed AmpC positive results for PCR amplification techniques, 4 were positive for *Escherichia coli* and 1 was positive for *Klebsiella pneumoniae*. PCR amplified DNA bands from clinical isolates were identified. The amplified bands were interpreted with 1000bp ladder.

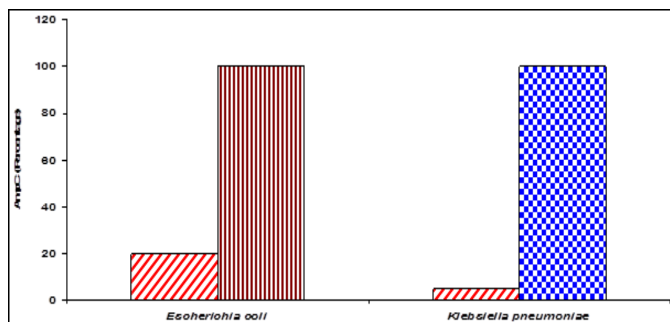


FIG. 8: PERCENT PREVALENCE OF AMPC GENE IN *ESCHERICHIA COLI* & *KLEBSIELLA PNEUMONIAE* STRAINS

DISCUSSION: Out of total 40 isolates examined for identification and characterization, 20 isolates of *Escherichia coli* 15 (75%) were positive for all tests and 5 (25%) were negative results for the same, while in case with 20 isolates of *Klebsiella pneumoniae* tested for the same as *E. coli*, 9 (45%) were positive for all tests and 11 (55%) were negative for the same, hence the clinical isolates which showed positive results for all the tests were further evaluated.

Further, the antibiotic sensitivity pattern carried out by Kirby Bauer disc diffusion method using Muller Hinton agar plates to detect Multi-drug resistant strain.

Out of 15 *E. coli* isolates tested for antibiotic sensitivity, 6 (40%) showed resistance to a minimum of 11 antibiotics and a maximum of 16 antibiotics, while 9 (60%) showed sensitivity to a minimum 7 and maximum of 11 antibiotics tested for the same. Of the 9 isolates of *Klebsiella pneumoniae* tested for sensitivity, 3 (33.33%) showed resistance of minimum 13 and maximum of 15 antibiotics, while 6 (66.66%) were sensitive to minimum 5 and maximum of 9 antibiotics tested for the same as compared with the study.

The gram negative isolates were sensitive to cefipime and meropenem. Strains sensitive to cefoxitine, evaluated for the presence of AmpC gene. Boric acid as an inhibitor of AmpC β-lactamases enzyme renders the strains sensitive.

Positive strains of MDRs strain carried out for screening of AmpC β-lactamases producing strains using a modified disc test, utilizing boronic acid as an inhibitor of AmpC enzymes, disc sensitivity

testing performed on Muller Hinton medium using a standard 30 µg cefoxitin disc and a similar cefoxitin disc 30 µg supplemented with 400 µg of phenyl boronic acid. Out of total 9 resistant strains tested, 6 *E. coli* and 3 *Klebsiella pneumoniae* strains showed an increase of 5mm in the zone diameter for the cefoxitin-phenyl boronic disc as compared with the cefoxitin disc, which was interpreted as a positive test for the AmpC producing strain based on the phenotypic interpretation as compared with the study performed. Further, plasmid DNA was isolated from the phenotypic positive strains of *E. coli* and *Klebsiella pneumoniae* isolates by rapid alkaline extraction method on agarose gel electrophoresis. The presence of plasmid DNA on the gel was compared with DNA ladder in the first lane.

Based on the plasmid identification by agarose gel electrophoresis was further evaluated for the determination of AmpC by PCR amplification techniques. Of the 9 strains tested, 4 were positive for *E. coli* and 1 was positive for *Klebsiella pneumoniae*. The amplified bands was compared with 1000bp marker (ranging from 1000, 750,500 and 250 respectively). PCR amplified DNA bands from clinical isolates were seen in the rage of 250 bp and below. Hence, the prevalence was detected from the clinical samples of urinary tract infected patients as 5 isolates out of 40 isolates of both strains.

CONCLUSION: Worldwide surveillance of antimicrobial resistance pattern among urinary pathogens is important to determine the seriousness of nosocomial infections. Multidrug resistant strains are highly problematic which increases the mortality rate, clinical cost and resistant against higher antibiotics. Moreover the stable resistant transformants were detected, indicating the transfer of plasmid genes carrying the multidrugs resistance markers.

The accurate detection of plasmid-mediated AmpC gene might be helpful in understanding the molecular mechanism for altering a newer drug discovery model which leads the fight against UTI causing agents and improve clinical managements of such infections. There is a need for continued surveillance studies on common nosocomial infections, which establishes a baseline resistance pattern in different geographical areas in order to find way to prevent further increase in antibiotic resistance and limit the cost associated with it. This surveillance is helpful to formulate in altering drug therapy in different clinical situation.

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