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ANALYSIS OF MULTIPLE ANTIBIOTICS RESISTANT AND MOLECULAR EPIDEMIOLOGY OF *ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA*

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Multidrug-resistant, Hospital-acquired infection, Uro pathogens and Plasmid

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ABSTRACT: The present investigation aimed at the study of resistant plasmid patterns of multidrug-resistant *Escherichia coli* and *Pseudomonas aeruginosa* isolated from hospital-acquired infection in urinary tract site are causes serious public health hazard throughout the world. A total of 100 urine samples were collected from patient admitted to the hospital. The antibiotic sensitivity of isolated uropathogens was performed by Kirby- Bauer method. The plasmid profiles of multidrug-resistant isolates were extracted by the alkaline lysis method. The multiple antibiotic-resistant indexes of uropathogens were calculated and phylogenetically determined. Among 100, 23 urine samples were positively isolated and identified. The *Escherichia coli* and was found to be predominantly followed by *Pseudomonas aeruginosa*. The multiple antibiotics resistant index was calculated as 0.75 and 0.464 respectively for *Escherichia coli* HAUTI8, and *Pseudomonas aeruginosa* HAUTI18, and these strains were phylogenetically identified. The Plasmid profile of *Escherichia coli* and *Pseudomonas aeruginosa* indicates wide ranges of plasmid among the resistant strains between 23,130 to 2027 base pairs. As a result, multidrug-resistant was an emerging disseminated clinical problem rapidly enhancing throughout the world.

INTRODUCTION: Hospital-acquired infection in urinary tract sites emerge over the past few decades has become a global health problem which can cause high morbidity and mortality throughout the hospital patients. Previous studies reported that urinary tract infection could occur in both males and females of any age, with bacterial counts as low as 100 colony forming units per millimeter in urine [1].

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The urinary tract infection caused by pathogenic bacteria that occur varies among different age groups of both male and female, catheterization, hospitalization, and previous exposure to all used common antibiotics [2].

The most common urinary tract infection caused by bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus* sp, and *Staphylococcus aureus* showed 90% urinary tract infection [3]. The most common Urinary tract infection was caused by *Escherichia coli* and *Pseudomonas aeruginosa* associated with symptomatic and asymptomatic bacteria in both sexes. The fourth leading cause of death occurred by a hospital-acquired infection in the urinary tract site. The main factor predisposing to urinary tract

infection has been attributed to poor personal hygiene, cultural habit imposition, and drug-resistant of isolates [4].

The mechanism for resistance has been reported for currently available all antimicrobial agents [5]. In India, 10-30% of patients are admitted to hospitals or nursing homes are associated with urinary tract infection. The Urinary tract infection has serious implications against multiple drug-resistant bacteria mediated by multiple drug-resistant plasmids [6]. The determination of plasmid is very useful for studying epidemiology. The *Escherichia coli* and *Pseudomonas aeruginosa* from urinary specimens are known to isolate plasmids of different molecular sizes, which can be horizontally transferred to other bacteria [7].

The spreading of multiple drug-resistant *Escherichia coli* and *Pseudomonas aeruginosa* is detected by using regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs, making policy decisions, and assessing the effectiveness of both. Indeed over a few years, the application of molecular techniques for isolation and differentiation of bacterial isolates in hospitals has provided a set of powerful new tools that can augment both epidemiological investigations and patient treatment [8]. The objective of this work was carried out to determine the resistant plasmids of multidrug-resistant *Escherichia coli* and *Pseudomonas aeruginosa* isolated from hospital-acquired infection in the urinary tract site.

MATERIALS AND METHODS:

Sample Collection and Handling: The urine samples were collected from patients of K.A.P. Vishwanatham Government College located at Tiruchirappalli, Tamil Nadu, India, with following sign and symptoms such as frequency of urination, inflammation, retention of urine, burning, fever, and chills of urinary tract infection patients. A total of 100 urine samples were collected and processed within 24 h by microbiological analysis.

Isolation and Identification of Clinical Pathogens from Urine Specimens: The isolation and identification urobacteria were analyzed by indole production test, Methyl red and Voges-proskauer test, Citrate utilization test, Catalase test, Oxidase test, nitrate reduction test and Triple sugar iron agar test. The UTI bacteria were characterized by EMB agar, MacConkey agar, and Blood agar.

Antibiotic Sensitivity Pattern of Clinical Isolates: According to the guidelines of the Clinical Laboratory Standard Institute, Multidrug resistance was detected by using the Disc Diffusion test, which was performed on Muller- Hinton agar medium. The test bacteria were inoculated by means of the swab method. After 10-15 min, selected antibiotics disc were placed over the bacterial growth with equal distance. The plates were incubated at 37 °C for 24 h. After incubation, the zone of inhibition was considered as an indicative of drug resistance or sensitive to the bacterial growth

Analyses of MAR Index: Based on the antibiotic sensitivity pattern of clinical isolates**,** multiple antibiotics resistance index was calculated by using the following formula:

MAR Index = Number of antibiotics to which the isolate was resistant / Total number of antibiotics tested

Plasmid Profile Analysis: Pure isolates of all bacteria strains were inoculated into the nutrient broth and incubated overnight. Resistant plasmid DNA was isolated by Birnboim and Dolly by the alkaline lysis method. The DNA was purified by phenol extraction and ethanol precipitation method and stored at -20 °C. The samples were run 0.8% agarose gel and stained by ethidium bromide. The DNA molecular weight marker was loaded along with the samples and electrophoresed. After electrophoresis, the stained gel was exposed under UV light transilluminator to visualize the bands of plasmid by using a gel documentation system.

Curing of the Plasmids: Curing of the Plasmid was done by Akinjogunla and Enabulele to determine whether a plasmid encodes a trait that codes for antibiotics resistance or multi-resistance. The isolates that showed multiple resistances to different antibiotics due to plasmid bands were subjected to plasmid curing. 0.1 mg/ml of ethidium bromide was added 100 ml of Luria Bertaru broth and was autoclaved at 121 °C at 15 psi for 15 min. An overnight culture of the sample was standardized according to 0.5 McFarland standards, and 0.5ml from the standardized solution was taken

using Pasteur pipette into the 100 ml sterile Luria Bertaru broth. The solution was incubated at 37 °C for 4 h. After incubation, the isolates were re-inoculated into a sterile nutrient broth and incubated for 24 h.

Phylogenetic Tree Construction: The reference sequence required for comparison was downloaded from the Genbank using BLASTN sequence-based retrieval system from Genbank (URL http://www.ncbi.nlm.nih.g) using our sequence as the query. Based on the sequenced data, the phylogenetic tree was constructed using the bioinformatics tool MEGA 5.05 for aligning the sequences by the Neighbor-joining method.

Nucleotide Sequence Accession Number: The 16S rRNA sequences for the *Escherichia coli* (HAUTI8) and *Pseudomonas aeruginosa (*HAUTI18) have been deposited in Gene Bank by using sequin (http://www.ncbi.nlm.nib.gov/genebank)

RESULTS:

Prevalence of UTI: A Total of 100 midstream urine specimens were collected from clinically challenged hospital-acquired infectious people in the age ranges between 1 -75. Among 100, 77 samples were found to be negative, and 23 samples were obtained positive results in **Table 1**. **Table 1** indicates the highest number of patients with hospital-acquired infection in the urinary tract site was found within the age range of 41-50, followed by the age range 31-40.

TABLE 1: DISTRIBUTION OF URINE SPECIMENS

Age Factor	Number of Urine Samples Collected	Number of Positive Samples
1-10	10	-
11-20	12	-
21-30	14	2
31-40	11	6
41-50	15	9
51-60	16	4
61-70	12	1
70-75	10	1
Total	100	23

Screening of Urobacteria: Among the 100 urine samples, 23 urine specimens were found to be positive, whereas 77 urine specimens were found negative. According to Bergey's manual of systematic bacteriology, cultural characterization and biochemical identification of pathogenic bacteria were isolated and identified. Among the bacterial isolate, 2 genera belong to Gram-negative rod-shaped bacilli. The dominant bacterial isolate of *Escherichia coli* showed that translucent greenish colonies on EMB agar and also ferment lactose to produce pink color on MacConkey agar. The biochemical properties of *Escherichia coli* exhibited to be positive in indole, methyl red, nitrate reduction, and catalase test. *Escherichia coli* showed acid and gas production in the Triple sugar iron agar test. *Pseudomonas aeruginosa* exhibited beta hemolysis on blood agar, whereas in MacConkey agar *Pseudomonas aeruginosa* found as non-lactose fermenter. *Pseudomonas aeruginosa* positively found in citrate, nitrate reduction, catalase, oxidase and also showed no triple sugar iron utilization. *Escherichia coli* was found to be the most predominant uropathogen isolated from the patients with UTI (n=17 samples) followed by *Pseudomonas aeruginosa* (n= 6 samples). Among 23 positive urine samples, the frequency of Gram-negative isolates *Escherichia coli* found in 73.91%, and *Pseudomonas aeruginosa* was 26.08%.

Antibiotics Resistant Pattern of *Escherichia coli*: According to the National Committee for Clinical laboratory standards (NCCLS) published guidelines for performing an antibiotic disc diffusion method on Mueller Hinton agar medium. Twenty-eight standard antibiotics disc were tested against *Escherichia coli* isolates. **Table 2** shows the antibiotics resistant pattern of *Escherichia coli*. All the isolated *Escherichia coli* showed 100% of resistance to ampicillin, aztreonam, and cefotaxime followed by cephalothin (76.47%), cefixime (70.58%), cefoxitin (64.70%) and ceftazidime (64.70%). The least resistance occurred in co-trimoxazole (5.88%) and gentamycin (5.88%). The 100% sensitivity pattern of *Escherichia coli* was found in amikacin, ertapenem, imipenem and meropenem followed by amoxicillin-clavulanate (82.35%), gentamycin (70.58%) and cefotaxime clavulanate (64.70%).

Antibiotic-Resistant Pattern of *Pseudomonas aeruginosa*: The *Pseudomonas aeruginosa* was isolated from six urine specimens. The antibiotics of ampicillin, aztreonam, cefepime, cefixime, cefpirome, ceftazidime, cefuroxime, ceftizoxime and trimethoprim were 100% resistant to *Pseudomonas aeruginosa* followed by amoxicillin clavulanate (83.33%), cefoxitin (66.66%) and

cefpodoxime (66.66%). The least resistance occurred in cephalothin (33.33%). The 100% sensitivity of *Pseudomonas aeruginosa* found in amikacin, cefpotaxime, cefpotaxime clavulnate, ertapenem, imipenem, meropenem, gentamycin, levofloxacin, tetracycline, and ofloxacin. The least sensitivity was showed in ciprofloxacin (66.66%), norfloxacin (66.66%), and nitrofurotoin (50%). The results of the antibiotics resistant pattern of *Pseudomonas aeruginosa* were observed in **Table 3**.

TABLE 2: DRUG RESISTANT PATTERN OF *ESCHERICHIA COLI*

S. no.	Tested Antibiotics	No. of resistant samples	% of resistant samples	No. of inter-mediate	No. of sensitive samples	% of sensitive samples
1	Amikacin	-	-	-	17	100%
2	Ampicilin	17	100 %	-	-	-
3	Amoxy/clav	-	-	3	14	82.35%
4	Aztreonam	17	100 %	-	-	-
5	Cefepime	9	52.94 %	5	3	17.64%
6	Cefixime	12	70.58%	4	1	5.88%
7	Cefoxitin	11	64.70%	3	3	17.64%
8	Cefpirome	10	58.82%	4	3	17.64%
9	Cefpodoxime	7	41.17%	6	4	23.52%
10	Ceftazidime	11	64.70%	4	2	11.76%
11	Ceftizoxime	5	29.41%	7	4	23.52%
12	Cefuroxime	10	58.82	6	1	5.88
13	Cephalothin	13	76.47%	2	2	11.76%
14	Cefotaxime	17	100%	-	-	-
15	Cefotaxime/clavulnate	-	-	6	11	64.70%
16	Ciprofloxacin	4	30.76%	5	8	47.04%
17	Co Trimoxazole	1	5.88%	5	11	64.70%
18	Ertapenem	-	-	-	17	100%
19	Gentamycin	1	5.88%	4	12	70.58%
20	Impienem	-	-	-	17	100%
21	Levofloxacin	5	29.41%	3	9	52.92%
22	Meropenem	-	-	-	17	100%
23	Moxifloxacin	2	11.76%	9	6	35.29%
24	Nitrofurantoin	-	-	7	10	58.82%
25	Norfloxacin	8	47.04%	3	6	35.29%
26	Ofloxacin	3	17.64%	6	8	47.04%
27	Tetracycline	2	11.76%	11	4	23.52%
28	Trimethoprim	6	35.29%	6	5	29.47%

TABLE 3: DRUG RESISTANT PATTERN OF *PSEUDOMONAS AERUGINOSA*

S. no.	Tested Antibiotics	No. of resistant samples	% of resistant samples	No. of inter-mediate	No. of sensitive samples	% of sensitive samples
1	Amikacin	-	-	-	6	100%
2	Ampicilin	6	100 %	-	-	-
3	Amoxy/clav	5	83.33%	1	-	-
4	Aztreonam	6	100 %	-	-	-
5	Cefepime	6	100 %	-	-	-
6	Cefixime	6	100%	-	-	-
7	Cefoxitin	4	66.66%	2	-	-
8	Cefpirome	6	100%	-	-	-
9	Cefpodoxime	4	66.66%	2	-	-
10	Ceftazidime	6	100%	-	-	-
11	Ceftizoxime	6	100%	-	-	-
12	Cefuroxime	6	100%	-	-	-
13	Cephalothin	2	33.33%	4	-	-
14	Cefotaxime	-	-	-	6	100%
15	Cefotaxime/clavulnate	-	-	-	6	100%
16	Ciprofloxacin	-	-	2	4	66.66%
17	Co Trimoxazole	-	-	6	-	-

18	Ertapenem	-	-	-	6	100%
19	Gentamycin	-	-	-	6	100%
20	Impienem	-	-	-	6	100%
21	Levofloxacin	-	-	-	6	100%
22	Meropenem	-	-	-	6	100%
23	Moxifloxacin	-	-	2	4	66.66%
24	Nitrofurantoin	-	-	3	3	50.00%
25	Norfloxacin	-	-	2	4	66.66%
26	Ofloxacin	-	-	-	6	100%
27	Tetracycline	2	-	-	6	100%
28	Trimethoprim	6	100%	-	-	-

Multiple Antibiotics Resistant Index (MARI): Among the total population of bacterial isolates resistant were determined by the antibiotic sensitivity test. Based on the antibiotic sensitivity of clinical pathogens were reveals that 73.91% of isolates were found to be Multidrug-resistant (MDR). Among the tested bacterial populations, high multiple antibiotic-resistant indexes were found in *Escherichia coli* HAUTI8 as 0.75. *Pseudomonas aeruginosa* HAUTI18 was observed in the range of 0.464, and the results were shown in **Fig. 1**.

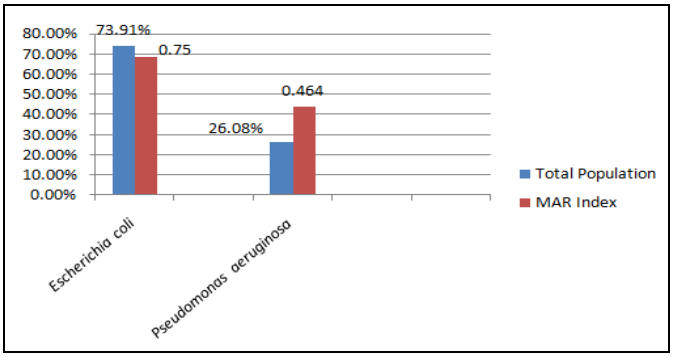

FIG. 1: DISTRIBUTION OF MULTI DRUG RESISTANT BACTERIA

Plasmid Profile of Clinical Isolates: Plate 1 shows the plasmid profile of multidrug-resistant bacteria was analyzed by alkaline lysis and electrophoresis methods. The lane M was marker consists of six bands ranging from 23, 130 bp – 2027 bp. Lane 1-17 corresponding to *Escherichia coli*. The plasmid analysis reveals that there were detectable plasmids in 14 isolates out of the 17 selected multi drug-resistant *Escherichia coli*. Among the 14strains, 12 of the isolates possessed single plasmid ranges 23, 130 bp. No plasmids were harvested from *Escherichia coli* isolates designated as 3, 5 & 16. Plasmid profile of lane 8 and 12 showed the presence of multiple bands indicates that these isolates are suspected to be highly resistant to antibiotics. Lane 18-23 corresponding to *Pseudomonas aeruginosa* reveals that there was detectable plasmid in 4 isolates possessed single plasmid out of the 6 strains, and lane 20 and 22 were no plasmids.

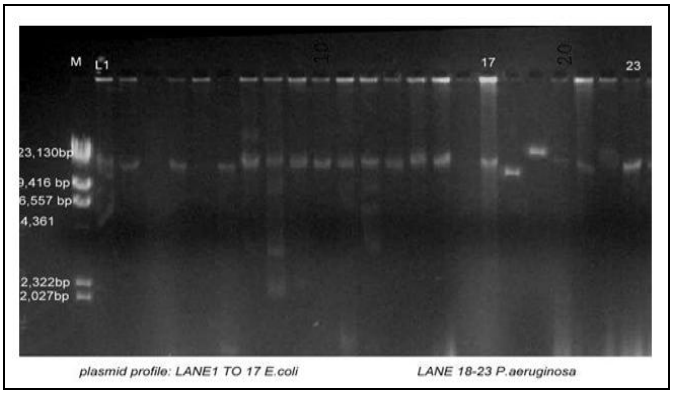

PLATE 1: PLASMIDS PROFILE ANALYSIS OF *ESCHERICHIA COLI & PSEUDOMONAS AERUGINOSA*

Molecular Characterization and Phylogenetic Analysis: The genomic DNA was extracted, and PCR amplification of almost full-length16Ss rRNA gene was carried out with eubacteria universal primer set –U1 (5'-CCAGCAGCCGCGGTA ATACG -3') and reverse primer U2 (5'-ATCGGCTACCTTGTTACGACTTC-3') [9].

The molecular characterization of *Escherichia coli* (HAUTI 8) showed 1300 base pairs, and *Pseudomonas aeruginosa* (HAUTI 18) showed 1400 base pairs. The *Escherichia coli (*HAUTI 8) and *Pseudomonas aeruginosa* (HAUTI 18) were deposited in NCBI, and the GenBank accession number of *Escherichia coli (*HAUTI8) was MG890199, and *Pseudomonas aeruginosa* (HAUTI18) was MG371997. **Fig. 2** indicates the phylogenetic analysis of the 16S rRNA sequence of *Escherichia coli* (HAUTI 8) was closely related to *Escherichia coli* RREC III and *Escherichia coli* TRB50. The *Pseudomonas aeruginosa* (HAUTI 18) was closely related to *Pseudomonas aeruginosa* D1 and *Pseudomonas aeruginosa* NO5.

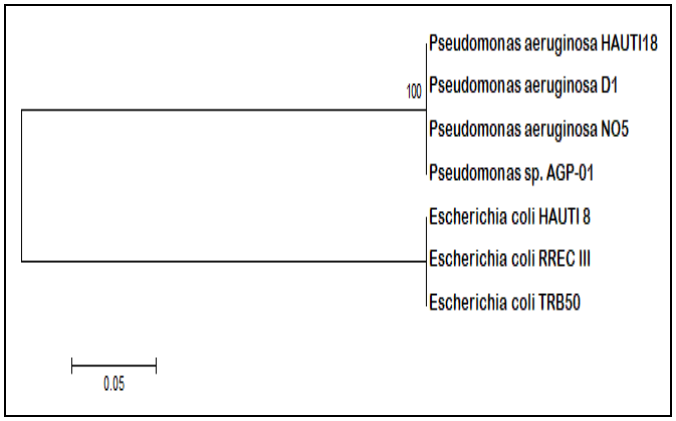

FIG. 2: EVOLUTIONARY RELATIONSHIPS OF *ESCHERICHIA COLI* (HAUTI8) *AND PSEUDOMONAS AERUGINOSA* (HAUTI18)

DISCUSSION:

Screening of Urobacteria: The present findings represent that a total of 100 samples were analyzed from a hospital setting. Twenty-three samples were found to be the site of hospital-acquired infection in the UTI site. Urinary tract infections are the most common type of hospital-acquired infection in community practice. Worldwide, about 150 million peoples are diagnosed with the urinary site of hospital-acquired infections occur in each year [10]. For diagnosis, urine samples were collected, and they found less than one type of bacterial pathogen. These findings were in good agreement with other workers represent that UTI is nothing but bacteria found in the urine (bacteriuria) and described as the growth of a single pathogen of $>10^5$ colony-forming units/ml from properly collected urine specimens [11].

According to Bergey‘s manual of systematic bacteriology, gram-negative bacterial populations were found to be at a high level than the gram-positive bacteria population. A total of two genera belonged to the bacterial population, such as *Escherichia coli* and *Pseudomonas aeruginosa.* The frequencies of most common nosocomial pathogens in our study were *Escherichia coli* (73.91%) and *Pseudomonas aeruginosa* (26.08 %). were the predominant bacteria found in hospitalized patients. The results of the current study were in accordance with other investigators who recorded nosocomial infection caused by *Escherichia coli* and *Pseudomonas aeruginosa* in UTI site [12].

Antibiotics Resistant Pattern of *Escherichia coli*: In several decades, multidrug-resistant bacteria pathogens have been created a constant problem in clinical practices. Later, they have been led to develop the emergence of multidrug-resistant bacteria to cause serious illness in a hospital setting. Out of 28 broad-spectrum antibiotics tested against 17 urine isolates of *Escherichia coli* and 6 urine isolates of *Pseudomonas aeruginosa.* The National Committee for Clinical laboratory standards (NCCLS) published guidelines used to screen the resistant bacteria by antibiotic disc diffusion test performed on Mueller Hinton agar medium.

Escherichia coli is one of the most common causative agents of hospital-acquired infection in the UTI site. Multidrug-resistant utmost importance for the clinical impact of gram-negative *Escherichia coli.* In our findings, *Escherichia coli* was highly resistant to ampicillin, aztreonam and cefotaxime followed by cephalothin, cefixime, cefoxitin, and ceftazidime and have no resistance was found in *E. coli* against amikacin, ertapenem, imipenem and meropenem. Our result was supported with other studies, high rates of resistance were found with ampicillin (91.66%), cefuroxime (82.29%), cefotaxime (79.16%) cefepime (67.7%) and ceftazidime (63.54%) among the *Escherichia coli* isolates. However, imipenem (0% resistance), meropenem (0%), amikacin (75.2%) and nitrofurantoin (26.04%) appeared to have retained higher sensitive activity [13]. Regrettably, the emergence of *Escherichia coli* was highly resistant to cefotaxime, cephalothin, ceftazidime, and other cephalosporin groups of antibiotics that have been documented seriously. *Escherichia coli* resistant to almost all currently used antibiotics may emerge in the future [14]. It has necessitated that the worldwide monitoring of multidrug-resistant producing *Escherichia coli* is urgently warranted.

Antibiotics Resistant Pattern of *Pseudomonas aeruginosa*: It is an opportunistic pathogen to cause hospital-acquired infection, with its ability to develop resistance to multiple classes of antibiotics, especially ampicillin and cephalosporin group of antibiotics [15]. In our present study, *Pseudomonas aeruginosa* was isolated from six urine specimens and were resistant highly to ampicillin, aztreonam, cefepime, cefixime, cefpirome, ceftazidime, cefuroxime, ceftizoxime, and trimethoprim

followed by amoxicillin clavulanate, cefoxitin and cefpodoxime. *Pseudomonas aeruginosa* is a uniquely problematic nosocomial pathogen and is naturally resistant to β-lactam, including broad-spectrum cephalosporins ampicillin, amoxicillin, ceftriaxone, ceftazidime and ciprofloxacin [16]. The cephalosporin groups of antibiotics, especially the third generation, has been documented for gram-negative bacterial treatment [17]. Among the tested antibiotics, ceftriaxone, ceftazidime, and cefotaxime were resistant to (60-100%) all the tested bacterial pathogens. It is similar to the study done by another investigator [18]. Antibiotic sensitivity reveals that the high degree of resistance (50 to 100 %) was reported against cephalosporin groups of antibiotics, monobactam, and ampicillin and aztreonam. This is agreed with other workers [19].

Determination of Multiple Antibiotics Resistant Index (MARI): The spread of bacterial resistance in a given population was measured by multiple antibiotics resistance (MAR) index whose value, if greater than 0.20, implied that such bacterial strains originated from an environment having several antibiotics. The obtained MAR indices indicated the exposure of a large proportion of the isolated bacteria to numerous antibiotics. The highest multiple antibiotic-resistant indices (MARI) for *Escherichia coli* HAUTI8 were 0.75. The overall rate of resistance against *Escherichia coli* was worldwide reported, which was similar to this study [20]. These bacteria are common environmental organisms which act as an opportunistic pathogen in clinical cases where the defense system of the patient is compromised to broad-spectrum antibiotic resistance, mainly penicillin and cephalosporins [21].

Plasmid Profile Analysis of Uropathogens: The spreading of resistant antibiotics coupled with the transmissibility of resistant determinants mediated by the plasmid. The emergence of plasmid-mediated multidrug-resistant has been increased in worldwide. A resistant plasmid that carries one or more antibiotic-resistant genes is currently recorded in cephalosporins, fluoroquinolones and aminoglycosides antibiotics that are used in clinical setting [22]. The result of this study showed that antimicrobial-resistant plasmids were harbored with hospital-acquired bacterial infection pathogens. Over expression of plasmids that confer resistant to many broad spectrum cephalosporin antibiotics including cefotaxime, ceftazidime and ceftriaxone due to hospital infection in UTI site are caused by Enterobacteriaceae family group of bacteria namely *Escherichia coli*, and *Pseudomonas aeruginosa* [23]. As compared to other workers, *Pseudomonas aeruginosa* is a plasmid-mediated resistance to various antibiotics has been documented [24]. Plasmid encoded by bacteria described predominantly are common among Enterobacteriaceae family belongs to the genera of *Escherichia coli*, and *Pseudomonas aeruginosa* are a breed of multidrug resistance. The results were in agreement with another researcher who reported that multidrug resistance of *Escherichia coli* and *Pseudomonas aeruginosa* isolates caring plasmids have been documented [25].

Molecular Characterization and Phylogenetic Analysis: The evolutionary history of *Escherichia coli* HAUTI8 and *Pseudomonas aeruginosa* HAUTI18 were inferred using the Neighbor-Joining method [26]. The optimal tree with the sum of branch length = 0.48000000 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches [27]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method [28] and are in the units of the number of base differences per site. The analysis involved 7 nucleotide sequences. The codon positions included were 1^{st} + 2^{nd} + 3^{rd} + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 50 positions in the final dataset. Evolutionary analyses were conducted in MEGA 5.05 [29].

In this present observation, the resistant plasmid DNA was detectable in 18 (78.26%) multidrug-resistant of 23 isolates. The emergence of resistant plasmid described the widespread use of antibiotics and exposure of enteric flora with poor sanitation of peoples. The bacterial pathogens which showed multiple drugs resistant were found to harbor the plasmids with molecular sizes ranging from 23,130 base pair - 2027 base pair.

These studies agreed with previously described reports stated that isolates of UTI bacteria pathogens with multidrug-resistant profiles were found to be possessed multiple resistant plasmids with large sizes in the ranging of 6,557-23,130 base pairs [30, 31].

CONCLUSION: There is an emerging situation in an increase of hospital-acquired infection caused by antibiotic-resistant bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. This study has highlighted the emergence of the diverse plasmid profiles and widespread antimicrobial resistance patterns among clinical bacterial isolates *Escherichia coli* and *Pseudomonas aeruginosa* from hospital-acquired infection in urinary tract site. Therefore the uncontrolled use of antibiotics must be a priority. Thus, Public health policy on appropriate prescribing and use of antibiotics must be instituted and affected based on recent antibiogram tests. This study showed that the monitoring of plasmid-mediated antibiotic resistance and antimicrobial susceptibility testing was necessary to avoid treatment failure conditions in patients with hospital-acquired infection in the urinary tract site.

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CONFLICTS OF INTEREST: The authors declared that there is no conflict of interest.

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