

# Phenotypic and genotypic characterization of multi-drug resistance *Pseudomonas aeruginosa* isolated from urinary tract infections of non-catheterized and catheterized Chinese patients

## A descriptive study over 3 years

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### Abstract

Urinary tract infections (UTI) are commonest infections, especially in catheterized patients. It is responsible of mortality and morbidity among hospitalized patients. The objectives of the study were to demonstrate the virulence factors and their genes of multi-drug resistance *Pseudomonas aeruginosa* causing UTI. A total of 366 non-catheterized and 171 catheterized patients' (in whom the catheter was in > 48 hours duration) urine samples (one sample/patient) from both sexes were collected and processed. >10<sup>5</sup> colony forming unit was considered as *Pseudomonas aeruginosa* culture-positive. Antimicrobial susceptibility testing was done by the Kirby Bauer disc diffusion method (The Clinical and laboratory standards institute guidelines 2019). The virulence factors were detected by in vitro assay method and polymerase chain reaction was done to detect the resistance genes present in *Pseudomonas aeruginosa*. Biofilm production was detected by the microtiter plate method. Out of 537 urine samples a total of 280 (52%) were females and 257 (48%) were male patients. Out of 366 non-catheterized urine samples 42 (23.6%) grew *Pseudomonas aeruginosa* and out of 171 catheterized urine 23 (25.84%) grew *Pseudomonas aeruginosa*. All were multi-drug resistance strains. A total of 10 (23.80%), 42 (100%), 8 (19.05%), 24 (57.14%), and 36 (85.71%) produced the Metallo- $\beta$ -lactamases, AmpC- $\beta$ -lactamase, carbapenemase, strong biofilm, and twitching motility positive, respectively in non-catheterized urine samples. A total of 11, 34, 9, 28, and 37 were oxacillinases-23, multidrug efflux protein resistance, New Delhi metallo- $\beta$ -lactamase-1, Verona Integron-encoded MBL, and *Pseudomonas* specific enzyme gene detected in non-catheterized urine samples. A total of 8 (34.8%), 6 (26.01%), 4 (17.39%), 15 (65.2%), and 18 (78.26%) were produced Metallo- $\beta$ -lactamases, carbapenemase, AmpC- $\beta$ -lactamase, strong biofilm, and twitching motility positive, respectively in catheterized urine samples. A total of 6, 18, 4, 16, and 15 were oxacillinases 23, multidrug efflux protein resistance, New Delhi metallo- $\beta$ -lactamase-1, Verona Integron-encoded MBL, and *Pseudomonas* specific enzyme, respectively genes detected in catheterized urine samples. Biofilm formation and twitching motility showed correlation among culture-positive *Pseudomonas aeruginosa* strains from catheterized patients (Correlation coefficients = 6.2, 95% confidence interval: 5.4–7.2). A better hospital infection control practice and detailed investigation of the microevolution of *Pseudomonas aeruginosa* in UTI are needed.

**Abbreviations:** CAUTI = catheter-associated urinary tract infections, EDTA = ethylenediamine tetra acetic acid, MEXABR = multidrug efflux protein resistance, MBL = Metallo- $\beta$ -lactamases, MDR = multi-drug resistance, NDM = New Delhi metallo- $\beta$ -lactamase, OD = optical density, PCR = polymerase chain reaction, PSE = *Pseudomonas* specific enzyme, QS = Quorum sensing, UTI = urinary tract infections, OXA = oxacillinases, VIM = Verona Integron-encoded MBL.

**Keywords:** biofilm, catheterization, metallo- $\beta$ -lactamases, multi-drug resistance, *Pseudomonas aeruginosa*, urinary tract infections, virulence

### 1. Introduction

Urinary tract infections (UTI) are the leading and commonest infections, especially in patients with indwelling catheters. UTI is an important cause of mortality and morbidity among hospitalized patients.<sup>[1]</sup> The predisposing factors associated with UTI

are diabetes mellitus, immunosuppression, urine incontinence in orthopedic patients and neurologically affected patients, and insufficiency of the kidney among patients with the renal problems.<sup>[1,2]</sup> The incidence of multi-drug resistance (MDR) pathogenic organisms is on an alarming rise due to resistant gene dissemination by transposons. Among UTI *Pseudomonas aeruginosa* infections are

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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the cause of 35% of catheter-associated urinary tract infections (CAUTI) than *Escherichia coli* which has been reported to be only 20% among CAUTIs.<sup>[3]</sup> Since *Pseudomonas* species have an inbuilt intrinsic antibiotic resistance and their capability of developing new resistance during antibiotic therapy makes these infections more complicated to eradicate. They also lead to more chronic and persistent infections.<sup>[4]</sup> Biofilm formation on the surface of the catheter is part of the infectious process.<sup>[5]</sup> Resistance to antibiotics, biocides, etc. is conferred by biofilm-associated proteins that protect the organism from the immune response from the host.<sup>[6]</sup>

Virulence of *Pseudomonas aeruginosa* is due to the production of extracellular, cell-associated virulence factors<sup>[7]</sup> type IV pili, LPS, flagella, etc.<sup>[8]</sup> Type II and III secreted extracellular virulence enzymes and extracellular polysaccharides alginate are important factors for biofilm formation and stability.<sup>[9]</sup> Biofilm which is an extracellular virulence factor is regulated by Quorum sensing (QS) which regulates the organism's capability to colonize effectively in humans.<sup>[10]</sup>

The antibiotic resistance mechanism of *Pseudomonas aeruginosa* includes decreased expression of opr D porin leading to less antibiotic permeability overexpression of MEXAB-oprM pump leading to increased antibiotic efflux, production of enzymes such as  $\beta$  lactams and aminoglycosides. A mutation is a combined mechanism leading to MDR in *Pseudomonas* species.

The virulence genes and phenotypes of *Pseudomonas aeruginosa* in biofilm formation during UTI are poorly understood. Here in our study, we demonstrate various virulence factors of *Pseudomonas aeruginosa* urethral isolate showing the presence of virulence factors such as biofilm, motility, QS, and alginate associated with MDR of *Pseudomonas aeruginosa* from UTI infections.

## 2. Materials and methods

### 2.1. Ethics approval and consent to participate

The designed protocol (AEH1584 dated January 1, 2017) was approved by the Tongji University review board. The study follows the law of China and the V2008 Declarations of Helsinki. Written informed consent for participation in the study has been obtained.

### 2.2. Sample collections

A total of 366 urine samples are collected from patients with urinary tract infections admitted to Chinese hospitals from January 3, 2018 to January 15, 2020. One sample per patient was collected. Clean catch mid-stream urine samples were collected via catheter port in the sterile container after clamping the catheter. Before collection, the catheter was cleaned with 70% isopropyl alcohol. After half an hour 1 to 2 mL of urine sample was aspirated using a sterile syringe into a sterile container. Samples collected from both sexes from patients of various wards such as medical, obstetrics and gynecology, intensive care units, surgery, and orthopedic were included in the study. A total of 171 catheter samples were collected from patients in whom the catheter was in more than 48 hours duration. All the urine samples collected from the catheter were transported to microbiology laboratories without further delay. All the specimens were processed according to standard protocol. All the urine samples were inoculated into cysteine lactose electrolyte deficient agar. Then, incubated for 18 to 24 hours at 37°C. Colony counts are likely to give a clear picture of normal urethral flora and pathogens. A colony count of  $> 10^5$  colony forming unit was taken for processing and identification. A single type of bacterial infection is mostly observed in nearly 96% of UTI.

### 2.3. Antibiotic sensitivity testing

Antimicrobial susceptibility testing was done by the Kirby Bauer disc diffusion method. The inoculum was prepared to match the 0.5 Mc Farlands turbidity standard. Following antibiotics are

used ceftazidime (30  $\mu$ g), cefuroxime (30  $\mu$ g), cefotaxime (30  $\mu$ g), gentamicin (10  $\mu$ g), nitrofurantoin (300  $\mu$ g), norfloxacin (10  $\mu$ g), ciprofloxacin (5  $\mu$ g), imipenem (10  $\mu$ g), meropenem (10  $\mu$ g), and piperacillin/tazobactam (100/10  $\mu$ g). This test was done following The Clinical and laboratory standards institute guidelines 2019.<sup>[11]</sup>

### 2.4. Phenotypic detection of resistance

To evaluate the Metallo- $\beta$ -lactamases (MBL) production among the *Pseudomonas aeruginosa* isolated, 2 Imp discs (10  $\mu$ g) and 2 MEM discs ( $\mu$ g), 10  $\mu$ L ethylenediamine tetra acetic acid (EDTA, 0.5M) was impregnated on 1 disc of each antibiotic. One EDTA disc and 1 without antibiotic were used as control. Mueller Hinton Agar plates were inoculated with inoculum and the disc was placed. MDR *Pseudomonas aeruginosa* with a 7 mm zone difference around Imp + EDTA, imp only disc was taken as MBL producers.<sup>[12]</sup>

### 2.5. Detection of biofilm

Biofilm production was detected by the microtiter plate method. Overnight culture of *Pseudomonas* species diluted, 1:100 in fresh Luria Bertani broth at 37°C for 24 to 48 hours. After incubation, 0.20% of crystal violet was added and kept at room temperature for 30 minutes. Wells were washed and fixed with 200  $\mu$ L of 95% ethanol for 10 minutes. Absorbance at 570 nm was taken to calculate the index of bacteria adhering to the surface.<sup>[13]</sup>

### 2.6. Motility assay

Bacterial motility was demonstrated by motility agar medium. Twitching motility is characteristic possessed by biofilm-producing *Pseudomonas* species. Colonies with twitching motility were stained with crystal violet and measured in mm.<sup>[14]</sup>

### 2.7. Detection of quorum sensing

A total of 100  $\mu$ L of the *Pseudomonas aeruginosa* strain inoculum were filled into the holes (0.7 cm in diameter) punched in 1.25% agar medium. After diffusion, the plates were overlaid with 4 mL of *Agrobacterium tumefaciens* NTL4 strain on plates with 30  $\mu$ g of 5-Bromo-4-chloride-3-indolyl  $\beta$ -D-galactose pyranosid (X-Gal). After overnight incubation at 30°C blue halos were considered positive and measured in mm.<sup>[15]</sup>

### 2.8. Molecular characterization of resistance gene

Polymerase chain reaction (PCR) was carried out to detect the resistance genes present in the pathogen (Table 1). DNA was extracted from colonies grown on agar plates and suspended in 20  $\mu$ L of deionized water. Vortex the mixture and incubated at 100°C for 5 minutes. Centrifuge the mixture to collect the supernatant for PCR. The reaction mixture contains 8.5  $\mu$ L of master mixture, 1  $\mu$ L each of forward and reverse primer, 1.5  $\mu$ L of gDNA template, 0.5  $\mu$ L of Taq polymerase, 7.5  $\mu$ L of nuclease-free water with total reaction set for 20  $\mu$ L. The PCR was performed in a thermocycler and the PCR products were analyzed in 1% agarose gel with 1  $\mu$ L of ethidium bromide using a 100 bp ladder visualized by UV trans illuminator and documented.<sup>[16]</sup>

PCR cycling conditions for oxacillinases 23 (OXA 23) and New Delhi metallo- $\beta$ -lactamase (NDM)-1 were denaturation at 94°C for 3 minutes, annealing at 55°C for 1 minutes, extension for 1 minutes 30 seconds at 72°C, and final extension for 7 minutes at 72°C for 32 cycles. PCR cycling conditions for MEX ABR were denaturation at 94°C for 3 minutes, 94°C for 30 seconds, annealing at 57°C for 45 seconds, extension for 1 minute at 72°C, and final extension for 7 minutes at 72°C for 32 cycles. PCR cycling conditions for *Pseudomonas* specific enzyme (PSE) were denaturation at 94°C for 2 minutes, 94°C for 20 seconds,

**Table 1**

**Genes detected by a polymerase chain reaction and their sequence.**

Genes	Sequence	BP
OXA-23 F	AGTATTGGGGCTGTGCT	453
OXA-23 R	AACTTCGGTGCCTATTTG	
MEX-A F	CTCGACCCGATCTACGTC	503
MEX -A R	GTCTTCACCTCGACACCC	
MEX -B F	TGTCGAAAGTTTTTCATTGATAG	280
MEX- B R	AAGGTCACGGTGATGGT	
MEX-R F	GAACTACCCCGTGAATCC	411
MEX-R R	CACTGGTCGAGGAGATGC	
PSE-F	AATGGCAATCAGCGCTTC	698
PSE-R	GCGCGACTGTGATGTATA	
VIM-F	GATGGTGTGGTCCGATA	391
VIM-R	CGAATGCGCAGCACCAG	
NDM-1 F	GCAGCACACTTCCTATCTCG	214
NDM-1 R	GTCCATACCGCCCATCTGT	

NDM = New Delhi metallo-β-lactamase, PSE = *Pseudomonas* specific enzyme, OXA = oxacillinases, VIM = Verona Integron-encoded MBL.

annealing at 48.8°C for 30 seconds, extension for 45 seconds at 70°C, and final extension for 7 minutes at 70°C for 29 cycles. PCR cycling conditions for Verona Integron-encoded MBL (VIM) were denaturation at 94°C for 2 minutes, 94°C for 20 seconds, annealing at 51°C for 30 seconds, extension for 30 seconds at 700 C, and final extension for 7 minutes at 700 C for 29 cycles.

**2.9. Statistical analysis**

All the variables’ dispersion and tendency were measured. The correlation was evaluated by regression analysis. The Fisher’s exact test or the Chi-square test was performed for categorial variables. All results were considered significant if *P*-value < .05. All data were analyzed using statistical software SPSS v20.0, IBM Corporation, Armonk, NY, USA.

**3. Results**

**3.1. Sample collections**

A total of 366 non-catheterized urine samples and 171 catheterized urine samples were collected. Out of 366 non-catheterized urine samples a total of 201 (54.9%) were females and 165 (45.08%) were male patients. Among 171 catheterized urine samples a total of 92 (53.08%) were males and 79 (46.1%) were females (Table 2). A total of 178 (48.63%) urine samples were culture positive out of 366 non-catheterized urine samples (Fig. 1) and a total of 188 (51.36%) urine samples showed no growth or insignificant bacteriuria. Out of 171 catheterized urine samples 89 (52.04%) showed culture positive and 82 (47.95%) showed insignificant bacteriuria or no growth (Fig. 2).

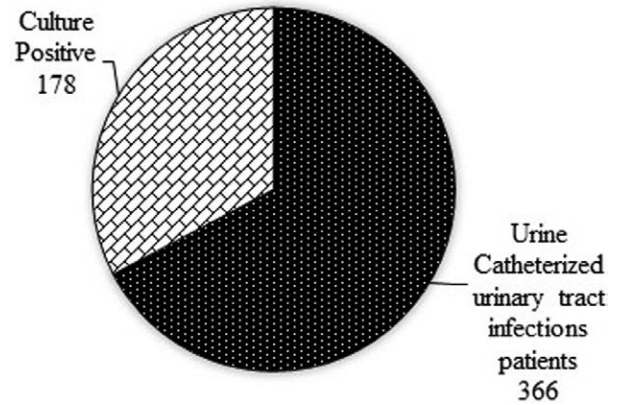
Out of 178 non-catheterized culture-positive urine samples, only 42 (23.6%) grew *Pseudomonas aeruginosa*. Similarly, out of 89 culture-positive catheterized urine samples, only 23 (25.84%) were grew *Pseudomonas aeruginosa* (Fig. 3, *P* = .7625, Fisher’s exact test).

**Table 2**

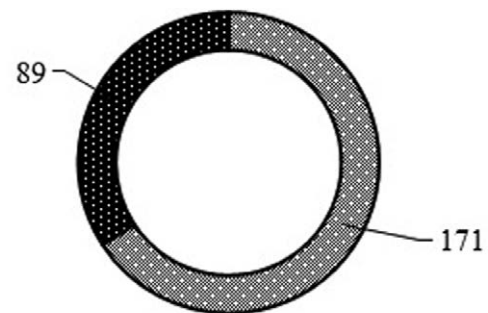
**Sex-wise distribution among catheterized and non-catheterized population.**

Sample	Male	Female	Comparison
Catheterized (N = 171)	92 (53.08%)	79 (46.1%)	0.0641 (Fisher’s exact test)
Non-catheterized (N = 366)	165 (45.08%)	201 (54.9%)	

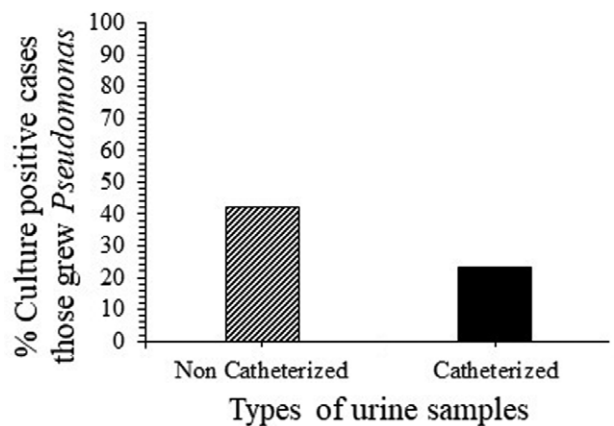
Variable are depicted as frequency (percentage).



**Figure 1.** Culture positive strains among non-catheterized urinary tract infections patients.



**Figure 2.** Culture positive strains among catheterized urinary tract infections patients.



**Figure 3.** Number of *Pseudomonades* isolated from non-catheterized and catheter-associated urinary tract infections patients.

### 3.2. Antibiotic sensitivity testing

The resistance pattern of *Pseudomonas aeruginosa* showed 100% resistance to cefotaxime, 94% resistance to ceftazidime, 84% resistance was observed for cefuroxime, and 54% resistance was observed for ciprofloxacin. A total of 26% resistance was noted for Piperacillin/tazobactam, 14% resistance seen in imipenem, 8% resistance observed for meropenem. The least resistance was observed for norfloxacin and nitrofurantoin with 6% and 4% respectively. In our study based on the antibiogram pattern, all the strains of *Pseudomonas* species are MDR strains (Table 3, Fig. 4).

### 3.3. Phenotypic detection of resistance

Out of 42 culture-positive *Pseudomonas aeruginosa* isolated from 366 non-catheterized urine samples, only 10 (23.80%) strains produced MBL. All 42 (100%) strains produced AmpC-β-lactamase. A total of 8 (19.05%) strains produced carbapenemase. Out of 23 culture-positive *Pseudomonas aeruginosa* strains isolated from 171 catheterized urine samples 8 (34.8%) showed MBL production, and 6 (26.01%) showed carbapenemase production. Only 4 (17.39%) strains showed AmpC-β-lactamase (Table 4).

### 3.4. Virulence factors detection

**3.4.1. Biofilm production.** Out of 42 culture-positive *Pseudomonas aeruginosa* strains from non-catheterized patients screened for biofilm production on absorbance optical density (OD) at 570nm was taken. Of 42 strains 24 (57.14%) were strong biofilm producers with OD 570 values 19.06 to 118.23.

A total of 18 (42.86%) were non-biofilm producers with OD 570 value 6.87 to 8.69 which was compared to OD 570 6.02 of crystal violet alone which served as control. Among 23 culture-positive *Pseudomonas aeruginosa* from CAUTI 15 (65.2%) produced strong biofilm with OD 570 value 22.03 to 117.24. 8 (34.8%) were non-biofilm producers with an OD 570 value of 5.84.

**3.4.2. Motility assay.** Twitching motility was observed in 36 (85.71%) out of 42 strains of MDR *Pseudomonas* species among the non-catheterized urine isolates. In catheterized UTI isolates of *Pseudomonas aeruginosa* showed 18 (78.26%) positive for twitching motility among 23 isolates obtained. Biofilm formation and twitching motility showed statistical significance and correlation coefficients were observed with values 6.2, confidence interval (95%) 5.4 to 7.2 among 23 culture-positive *Pseudomonas aeruginosa* from CAUTI.

**3.4.3. Detection of quorum sensing.** Out of 42 culture-positive *Pseudomonas aeruginosa* strains from non-catheterized patients, only 21 (50%) showed positive for QS by in vitro assay. Out of 23 CAUTI *Pseudomonas aeruginosa* isolates only 14 (60.86%) showed positive. These were not statistically significant. The results of virulence factors detection are presented in Table 5.

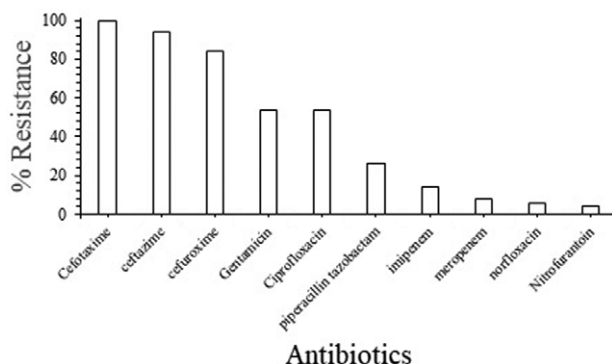
### 3.5. Molecular detection of resistance genes

Among the 42 UTI isolates from non-catheterized patients, only 11 (26.19%) showed the presence of the OXA-23 gene by PCR. A total of 6 (26.08%) showed the presence of the OXA-23 gene among CAUTI isolates. MEX-ABR gene was observed in 34 (80.95%) non-catheterized *Pseudomonas aeruginosa* isolates and 18 (78.26%) CAUTI isolated showed the presence of the MEX-ABR gene. NDM-1 gene was detected in 9 (21.42%) UTI isolates and 4 (17.39%) CAUTI isolates showed positive for the NDM-1 gene. VIM gene was detected in 28 (66.6%) non-catheterized *Pseudomonas aeruginosa* isolates and 16 (69.6%) CAUTI isolates showed the presence of the gene by PCR. A total of 37 (88.1%) non-catheterized *Pseudomonas aeruginosa* isolates and 15 (65.2%) CAUTI isolates were positive for the presence of the PSE (Table 6).

**Table 3**

**Antibiotic resistance pattern of non-catheterized and catheter-associated urinary tract infections of *Pseudomonas aeruginosa* isolates.**

Antibiotics	Resistance (%)
Cefotaxime	100
Ceftazime	94
Cefuroxime	84
Gentamicin	54
Ciprofloxacin	54
Piperacillin + tazobactam	26
Imipenem	14
Meropenem	8
Norfloxacin	6
Nitrofurantoin	4



**Figure 4.** Antibiotic resistance pattern of non-catheterized and catheter-associated urinary tract infections *Pseudomonas aeruginosa* isolates.

## 4. Discussion

*Pseudomonas aeruginosa* is of great importance as an opportunistic pathogen with multiple antibiotic resistance complicating treatment in infected patients. It is a threat to the hospital due to its survival capability in a most vivid environment in the hospital posing a nosocomial infection threat.<sup>[17]</sup> Intrinsic resistance possessed by this bacterium is an important characteristic feature.<sup>[18]</sup> In recent years MDR leads to a global health problem that needs an in-depth study to understand this bacterium.<sup>[19]</sup> In our study overall incidence of *Pseudomonas aeruginosa* infection were 23.6% in non-catheterized *Pseudomonas aeruginosa* isolates and 25.84% in CAUTI patients which is less compared to the study by Jaikaran et al,<sup>[16]</sup> who had reported 41% incidence rate. The age-wise comparison showed non-catheterized *Pseudomonas aeruginosa* isolates were mostly in the reproductive age of 25 to 40 years whereas CAUTI patients were in 45 to 60 years of age. Our study correlates well with Bagchi et al<sup>[20]</sup> studies also showed patients in age 60 years are more prone to infection with *Pseudomonas* species.

MDR strains of *Pseudomonas aeruginosa* in other studies were 63.79%. One of the important infections causing an agent of UTI.<sup>[21]</sup> Other studies suggest that higher MDR prevalence is due to the inappropriate use of antibiotics for treatment.<sup>[22]</sup> In our study, we observed 100% resistance to cefotaxime, 94% resistance to ceftazidime, 84% resistance to cefuroxime, and 54%

**Table 4****Phenotypic detection of resistance among non-catheterized and catheter-associated urinary tract infections isolates.**

Culture-positive urine samples	Metallo- $\beta$ -lactamases	AmpC- $\beta$ -lactamase	Carbapenemase	Comparison
Non-catheterized urinary tract infections isolate (N = 42)	10(23.80%)	42(100%)*	8(19.05%)	0.0014 ( $\chi^2$ -test)
Catheterized urinary tract infections isolates (N = 23)	8(34.8%)	4(17.39%)	6(26.01%)	
Comparison (P-value; Fisher's exact test)	.3928	<.0001	.5404	

\*Statistical significance.

Variable are depicted as frequency (percentage).

**Table 5****Virulence factors of non-catheterized and catheter-associated urinary tract infections isolates.**

Virulence factors	Non-catheterized urinary tract infections (N = 42)	Catheter-associated urinary tract infections (N = 23)
Biofilm production	24(57.14%)	15 (65.2%)*
Motility	36(85.71%)	18 (78.26%)*
Quorum sensing	21(50%)	14 (60.86%)

\*Statistical significance and correlation coefficients between biofilm formation and twitching motility were observed with a value of 6.2, with the confidence of interval (95%) 5.4–7.2.

Variable are depicted as frequency (percentage).

**Table 6****Resistance genes detected by a polymerase chain reaction of non-catheterized and catheter-associated urinary tract infections isolates.**

Culture-positive urine samples	OXA-23	MEXABR	NDM-1	VIM	PSE	Comparison
Non-catheterized (N = 42)	11 (26.19%)	34 (80.95%)	9 (21.42%)	28 (66.6%)	37 (88.1%)	0.9439 ( $\chi^2$ -test)
Catheter-associated urinary tract infections (N = 23)	6 (26.08%)	18 (78.26%)	4 (17.39%)	16 (69.6%)	15 (65.2%)	
Comparison (P-value; Fisher's exact test)	.9999	.9999	.7581	.9999	.0489*	

Variable are depicted as frequency (percentage).

\*Statistical significance.

NDM = New Delhi metallo- $\beta$ -lactamase, PSE = *Pseudomonas* specific enzyme, MEXABR = multidrug efflux protein resistance, OXA = oxacillinases, VIM = Verona Integron-encoded MBL.

resistance to gentamicin which coincides with the other studies. In our study 10/42 (23.8%) of the non-catheterized *Pseudomonas aeruginosa* isolates showed MBL, and 8/23 (34.78%) CAUTI isolates showed presence of MBL. Carbapenemase production was observed in 8/42 (19.05) of non-catheterized *Pseudomonas aeruginosa* strains and 6/23 (26.09%) of CAUTI isolates showed Carbapenemase production which is slightly less compared to 43% reported by Ochoa et al.<sup>[23]</sup>

Our data shows MDR strains of *Pseudomonas aeruginosa* showed higher biofilm production of 57.14% among non-catheterized *Pseudomonas aeruginosa* isolates and 42.86% in CAUTI isolates showed biofilm formation. Biofilm affects the therapy by forming physical and chemical resistance to antibiotic penetration in planktonic cells.<sup>[24]</sup> The results of the current study correlates with other studies of burrows 2012 and Subramanian et al, 2012 with high biofilm production among urinary isolates in their study.<sup>[25]</sup> MDR hampers the treatment and causes exacerbation threat in the pathogenesis of the strain.

Our study showed that 85.71% of non-catheterized *Pseudomonas aeruginosa* isolates with twitching motility and 78.26% of CAUTI isolates possessed twitching motility of more than 6 to 18.45 mm in diameter. These results suggest twitching motility is essential for the formation of biofilm structures in the MDR *Pseudomonas aeruginosa* strain. Our study results of motility are like those of Colvin et al<sup>[26]</sup> studies which reported twitching motility as an important factor for biofilm production. Statistical significance of twitching motility and biofilm formation was also observed in our study with a 6.2 correlation coefficient value. Twitching motility is an important factor in biofilm formation.

PCR analysis of the resistance gene showed 66.6% of the VIM gene which is slightly higher compared to the study by Zafer et al<sup>[27]</sup> who had reported 58.61% VIM gene in *Pseudomonas*

*aeruginosa*. PSE gene was observed in 88.1% of non-catheterized *Pseudomonas aeruginosa* isolates and 65.2% of CAUTI isolates which is less compared to the study by Cho et al<sup>[28]</sup> who had reported 84% of PSE gene present in *Pseudomonas aeruginosa*. CAUTI are more virulent than those of non-catheterized UTI.

Natural bacterial resistance towards antibiotics and detergents contributed by efflux systems are detected in our study with 50% of strains showing QS activity in non-catheterized *Pseudomonas aeruginosa* isolates and 60.86% among CAUTI *Pseudomonas aeruginosa* isolates which is less compared to studies which reported 100% in their study.<sup>[29]</sup> multidrug efflux protein resistance gene was reported in all the isolates by Al grawi et al<sup>[30]</sup> but our study showed 80.95% among non-catheterized *Pseudomonas aeruginosa* isolates and 78.26% in CAUTI isolates. NDM-1 was detected in 21.42% of non-catheterized *Pseudomonas aeruginosa* isolates and 17.39% of CAUTI isolates which is less than studies by Rahman et al who reported 29.23% of the NDM-1 gene in their studies. OXA-23 gene was found in 26.19% of non-catheterized *Pseudomonas aeruginosa* isolates and 26.08% in CAUTI isolates which is less compared to the study by Pragasam et al<sup>[31]</sup> who had reported 34.24% in their study.

In the limitations of the study, for example small sample size. Studies reporting descriptive results from a single institution (or area). The association of factors associated with catheterization and UTI are not evaluated.

## 5. Conclusions

Our study shows that carbapenem-resistant urinary isolates with the presence of multidrug, pan drug resistance genes raise an alarm in the spread of the notorious pathogen, especially in catheterized patients with associated comorbidities and the

mortality rate may increase. A better hospital infection control practice and detailed investigation on the microevolution of *Pseudomonas aeruginosa* in urinary tract infections and high warrant track of ever-increasing resistance are need of the hour to be focused for effectively controlling and reducing the spread of resistance strain.

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**Project administration:** Yanhong He.

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**Software:** Lamei Wu.

**Supervision:** Yanhong He.

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**Visualization:** Pingming Liao.

**Writing – original draft:** Huijian Yang.

**Writing – review & editing:** Huijian Yang.

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