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Molecular Detection of Class 1 Integron Gene and Antibiotics Susceptibility Patterns of *Pseudomonas* Species Isolated from Clinical Specimens

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ABSTRACT

Pseudomonas species is a common cause of health care acquired infection and also stand in second place in WHO critical list for antimicrobial resistance. Acquisition of antibiotic resistance gene in pathogenic bacteria has been a growing problem worldwide. The presence of resistance gene in class 1 integrons is associated with multi- drug resistance among *Pseudomonas* species. This study aimed to detect the class 1 integron and antibiotic susceptibility patterns of *Pseudomonas* species isolated from clinical specimens at No. (1) Defence Services General Hospital (1000-bedded) during the period of January to September 2020. The hospital and laboratory based descriptive study was conducted among the clinical samples received at Microbiology laboratory. Isolation, identification and antimicrobial susceptibility testing were performed by Vitek 2 automated systems. All *Pseudomonas* species were tested for the presence of class 1 integron by PCR. In this study, 77 *Pseudomonas* species isolated from various clinical specimens. According to the finding, the most common resistance was observed towards cefotaxime (97.4%), while resistance to amikacin was less observed among isolates (22%). Out of 77 *Pseudomonas* species isolates, 54 (70%) were multidrug resistant (MDR) according to CLSI 2020 guidelines. Among the multidrug resistance, 38 (95%) isolates were class 1 integron positive ($p<0.001$) and 16 (43.24%) were class 1 integron negative ($p<0.001$). Therefore, this finding indicates the strong association between the presence of class 1 integron and multidrug resistance. Therefore, integrons play an important role in acquisition and dissemination of antibiotics resistance genes among *Pseudomonas* species.

Keywords: *Pseudomonas* species, antimicrobial resistance, resistance gene, integron

1 Introduction

Pseudomonas is a gram-negative opportunistic pathogen that is an important cause of life-threatening infections associated with hospitalization. Nosocomial infections caused by *Pseudomonas* are often difficult to treat because this organism displays resistance to all, or almost all, commercially available antibiotics. *Pseudomonas* species also stand in second place in WHO critical list for antimicrobial resistance [1].

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Among various mechanisms involved in antimicrobial resistance, the acquisition of resistance genes by horizontal transfer mediated by integron play a crucial role. Although 3 major classes of integrons have been described in bacterial isolates, class 1 integrons have been found to be the most prevalent in clinical isolates of gram-negative bacteria including *Pseudomonas* species. Class 1 integrons carrying single or multiple gene cassettes, which confer resistance to aminoglycosides, β-lactams, chloramphenicol, carbapenems, and macrolides [2].

In No (1) Defence Services General Hospital (1000 Bedded), culture positivity of *Pseudomonas* species was 15% in 2018 and 11% in 2019 respectively. The isolation rate of multidrug resistance (MDR) *Pseudomonas* species was 30% in 2018 and 80% in 2019**.**

A study conducted in Tanta University Hospitals reported that 58.9% of *Pseudomonas* isolates were MDR and 45.3% were Class 1 integron positive [3]. Chen et al (2009) reported that 38% (27 of 71) of *Pseudomonas* isolated from Zhenjiang area of China carried class 1 integrons. Among integron-positive strains, 90.1% were MDR [4]. A study conducted in Northern Thailand revealed that 82% (41 of 50 MDR) *Pseudomonas aeruginosa* carried class 1 integrons [5]. In Southeast Iran, 40.4% of isolated *Pseudomonas* species were multidrug resistant and of which 95% carried class 1 integron [6].

The emergence of multidrug resistant strains is up surging leading to problematic control. In Myanmar, resistance to various antibiotics is common in clinical isolates, especially among *Pseudomonas* species. According to findings from previous studies conducted in No (1), Defence Services General Hospital (1000-bedded), multidrug resistance among *Pseudomonas* species is increased in alarming rate.

The rapid dissemination of antibiotic resistance genes among bacterial isolates is an increasing problem in infectious disease. Integrons play a key role in the expression and spread of antibiotic resistance genes. There are several studies showing class 1 integrons in clinically significant bacterial strains in European and Asian countries. In Myanmar, association between antibiotics resistance and presence of integrons have not yet been investigated in a large-scale study.

2 Materials and Methods

It was hospital and laboratory based descriptive study and it took a period of 9 months (from January 2020 to September 2020). A total of 2297 samples were received from patients attending at No (1) Defence Services General Hospital (1000-bedded). Samples were collected according to Clinical Laboratory Standard Institute (CLSI) Guidelines (M100 S30 2020).

2.1 Pseudomonas Species Identification and Antimicrobial Susceptibility Testing

All samples were cultured according to Standard Operation Procedure. After 24 hours incubation at 37°C, the plates were examined macroscopically and microscopically for colonies of *Pseudomonas species*. The VITEK 2 compact system was used for species identification and antimicrobial susceptibility testing of each isolate. *Pseudomonas* species isolates were stored in 20% glycerol at -20°C and colonies were sub-cultured onto the MacConkey agar plate at overnight incubation before the molecular work was performed.

2.2 Polymerase Chain Reaction

PCR was used to amplify the class 1 integron gene in *Pseudomonas* species. Bacterial DNA was extracted by boiling method and integrase genes *intI 1* was detected by using specific primers design to amplify the conserved regions of the respective genes. Primers (Int1F 5′-3′

AAGGATCGGGCCTTGATGTT and Int1R 5′-3′ CAGCGCATCAAGCGGTGAGC) 471bp were used. The reaction mixture was prepared using 2.5 µl of 10X ThermoPol Reaction Buffer, 0.5 µl of 10mM dNTPs, 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 0.125 µl of Taq DNA Polymerase, Template DNA 2 µl and add 18.875 µl of Nuclease free water. The samples were amplified in Applied Biosystem 7500 Fast Thermocycler instrument (Thermofisher, USA). Cycling program was as follows: preincubation at 94˚C for 5 min followed by 30 cycles of denaturation at 94˚C for 30 sec, annealing at 56˚C for 30 sec, extension at 72˚C for 30 sec and final extension at 72˚C for 7 min. Then, PCR products were separated by gel electrophoresis on 1.5 % agarose gels and were visualized under UV light.

Table 1: *Primer used in this study*

Name of the primer	Target	Amplicon	Sequence 5' to 3'
Int 1 Forward	Int 1 gene	471 bp	AAGGATCGGGCCTTGATGTT
Int 1 Reverse			CAGCGCATCAAGCGGTGAGC

3 Results

3.1 Distribution of Pseudomonas species

During study peroid, 688 bacterial pathogens were identified from 2297 various clinical samples received at Microbiology Laboratory. Out of 688 pathogenic bacteria, 77 (11.2%) were *Pseudomonas* species. *Pseudomonas* species contributed from wound swab sample were 18.1% (25 of 138 positive culture), 11.35% from urine (21/ 185), 11.73% from sputum (19 /162), 4.63% from blood culture (5/ 108), 7.37% from other specimens such as ascites fluid, catheter tip, CSF, tip, fluid, nasal discharge and pus (7/ 95) as show in (Table -1). Out of 77 *Pseudomonas* species, 42 (54.5%) were isolated from surgical wards, 23 (29.9%) were from medical wards and 12 (15.6%) were from ICU (Figure-1).

Table 2: *Distribution of Pseudomonas species in different types of samples*

Types of clinical samples	Bacterial pathogens from positive culture	*Pseudomonas* species (%)	
		Detected	Not Detected
		No (%)	No (%)
Wound swab	138	25 (18.1%)	113 (81.9%)
Urine	185	21 (11.35%)	164 (88.65%)
Sputum	162	19 (11.73%)	143 (88.27%)
Blood culture	108	5 (4.63%)	103 (95.37%)
Others	95	7 (7.37%)	88 (92.63%)
Total	688	77 (11.2%)	611 (88.8%)

3.2 Antibiotic susceptibility pattern of Pseudomonas species (N=77)

Resistance rates to various antibiotics were as follows: cefotaxime (97.4%), levofloxacin (71.4%), ciprofloxacin (64.9 %), gentamicin (59.7%), meropenem (57.1%), cefepime (54.5%)

and imipenem (51.9%) while the most effective antibiotic was amikacin and piperacillin/tarzobactem with a resistance rate of 22.1 % and 45.5% respectively (Figure-2).

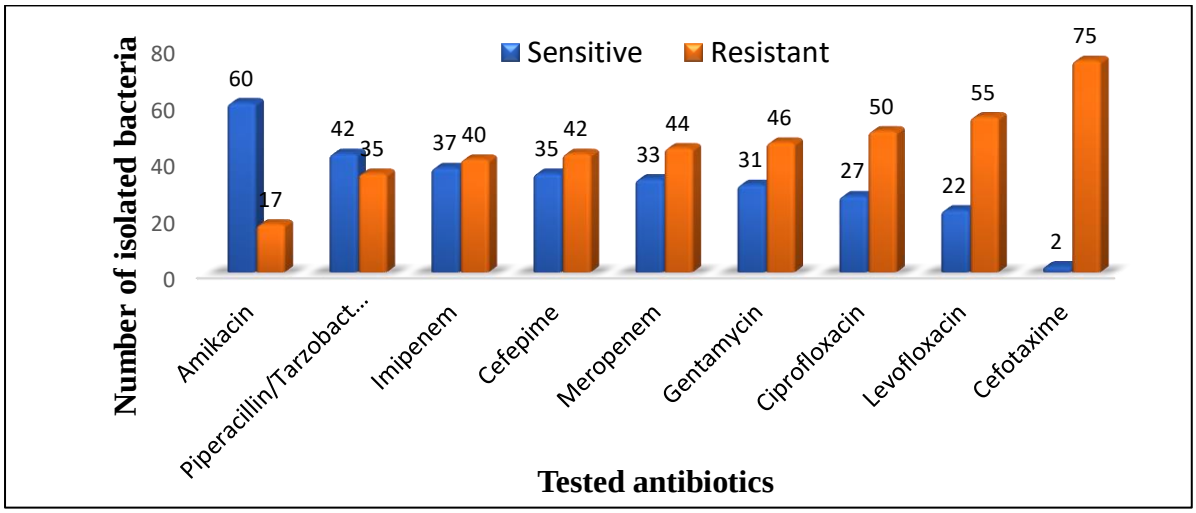

Figure 1: *Antibiotic susceptibility pattern of Pseudomonas species (N=77)*

3.3 Antibiotic susceptibility pattern of class 1 integron-positive and class 1 integron-negative Pseudomonas species

In the present study, 52% (40/77) isolated *Pseudomonas* species harbored class 1 integron. A significant correlation was obtained between the presence of integrons and resistance against cefepime, ciprofloxacin, levofloxacin, gentamycin, imipenem and meropenem ($P < 0.001$).

Table 3: *Antibiotic susceptibility pattern of class 1 integron-positive and integron-negative Pseudomonas species*

Antibiotics	**Class 1 integron positive**		**Class 1 integron negative**		***P* value**
	Sensitive No. (%)	**Resistant No. (%)**	**Sensitive No. (%)**	**Resistant No. (%)**	
Cefotaxime	1 (1.3%)	38 (49.4%)	1 (1.3%)	37 (48%)	0.985
Cefepime	8 (10.4%)	31 (40.3%)	27 (35.1%)	11 (14.2%)	**0.001**
Piperacillin/Tarzobactam	19 (25%)	20 (26%)	23 (30%)	15 (19%)	0.298
Ciprofloxacin	4 (5%)	35 (46%)	23 (30%)	15 (19%)	**0.001**
Levofloxacin	1 (1.3%)	38 (49.4%)	21 (27.3%)	17 (22%)	**0.001**
Amikacin	27 (35%)	12 (16%)	33 (43%)	5 (6%)	0.62
Gentamycin	6 (8%)	33 (43%)	25 (32%)	13 (17%)	**0.001**
Imipenem	11 (14%)	28 (36%)	26 (34%)	12 (16%)	**0.001**
Meropenem	32 (41%)	7 (9%)	12 (16%)	26 (34%)	**0.001**

3.4 Association between Class 1 Integron positivity and multidrug resistance Pseudomonas species

Of total 77 *Pseudomonas* species, 54 (70%) *Pseudomonas* species were MDR and 8 (10%) were PDR. Among the multidrug resistant *Pseudomonas* species, integron positive and integron negative isolates were 95% and 43.24% respectively. Therefore, significant association was found between class 1 integron positivity and multidrug resistance *Pseudomonas* species ($p < 0.001$) (Table-3).

Table 4: *Association between class 1 Integron positivity and multidrug resistant Pseudomonas species*

MDR	Integron positive	Integron negative	Total	*p* value
Positive	38 (95%)	16 (43.24%)	54	<0.001
Negative	2 (5%)	21 (56.76%)	23	
Total	40	37	77	

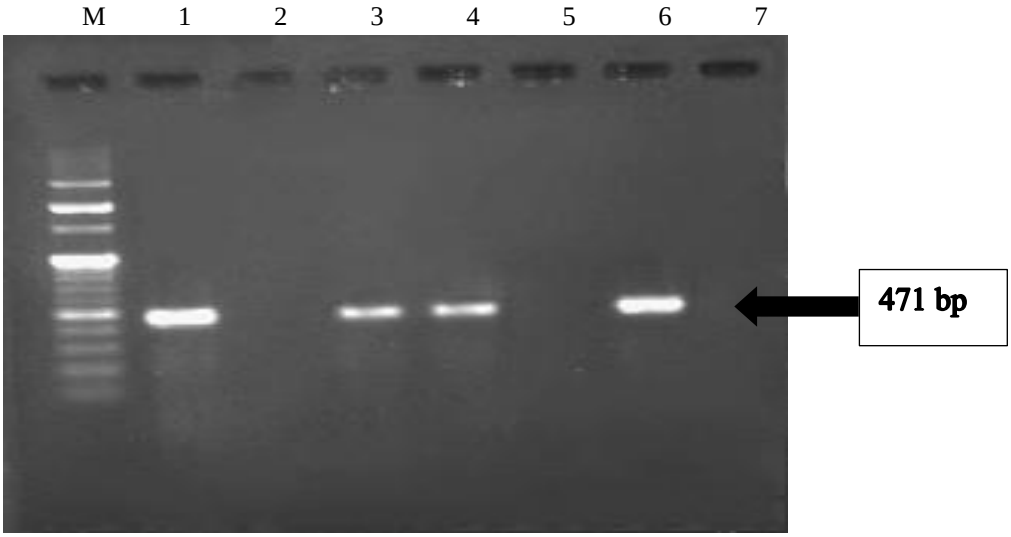

Plate 1: *Detection of class 1 integron in Pseudomonas species by PCR*

Lane M - 100bp DNA ladder, Lane 1,3,4,6 - Sample positive for *int1* gene at 471 bp, Lane 2 and 5 - Sample negative, Lane 7 - No template control

4 Discussion

Pseudomonas is one of the most important pathogens causing a wide range of infections in the hospital and health care setting. The spreads of resistance to antimicrobial agents and the emergence of multidrug resistance *Pseudomonas* have become a serious problem in the treatment of nosocomial infection worldwide. Resistance to antimicrobial agents can be originated from many resistance genes that present as gene casettes and transferred through integrons.

In the present study, highest number of *Pseudomonas* species was isolated from wound swab samples (18.1%) followed by a urine (11.35 %), sputum (11.73%), blood culture (4.63%) and 7.37% from other clinical specimens Table (2). The *Pseudomonas* infection of the wound might

cause sepsis, longer hospital stays, and increasing healthcare cost, and it also might affect the morbidity and mortality of an individual. *Pseudomonas* species were mostly isolated from surgical ward (55%, 42 of 77 *Pseudomonas* isolates), the high incidences of *Pseudomonas* infections happened in the surgical ward might be caused by exposures toward invasive procedures.

In this study, *Pseudomonas* species demonstrated highest resistance against cefotaxime (97.4%), followed by levofloxacin (71.4%), ciprofloxacin (64.9%), gentamycin (59.7%), meropenem (57.1%), cefepime (54.5%), imipenem (51.9%), piperacillin/tarzobactem (45.5%) respectively and showed higher sensitivity to amikacin (77.9 %) (Figure -2). The present study showed a high rate of resistance of isolates to cefotaxime (97.4%).

In the present study, 70% (54 of 77) *Pseudomonas* species were MDR, and 8 isolates showed resistance to all tested antibiotics (Pandrug-resistant (PDR)). Prevalence of MDR *Pseudomonas* species was gradually increased when compared with previous studies conducted in No. (1) DSGH (1000-Bedded). Prevalence of MDR *Pseudomonas* species among isolated *Pseudomonas* species were found to be 30% and 80% in 2018 and 2019 respectively. Therefore, routine antibiotic sensitivity test should be done to prevent the treatment failure and effective strategy on the limited and provident use of antipseudomonal agents should be established by hospitals, clinicians, clinical microbiologists, public health officials to convoy empirical therapy.

A study in Egypt, Iraq, Iran and China study showed that 45.3%, 35%, 95% and 38% of *Pseudomonas* were Class 1 integrons positive [3][7][6] [4]. In the present study, 52% (40 of 77) isolated *Pseudomonas* species harboured class 1 integron. A significant correlation was obtained between the presence of integrons and resistance against cefepime, ciprofloxacin, levofloxacin, gentamycin, imipenem and meropenem ($P < 0.001$) (Table2) and MDR (Table3).

5 Conclusion

These findings suggest that antibiotic-resistance genes captured by class 1 integrons in *Pseudomonas* isolates under constant antibiotic-selective pressure. The emergence of the high frequency of *int 1* gene in No (1) DSGH may explain a serious concern in the future. Therefore, it is important to perform antibiotic surveillance programs for appropriate empirical therapy and infection control practices.

6 Declarations

6.1 Ethical Consideration

Ethical clearance was obtained by Institutional review Board of DSMA.

6.2 Acknowledgements

We give our special thanks to all personnel who participated in this study.

6.3 Competing Interests

The authors declare that they have no conflict of interest.

6.4 Publisher's Note

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