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Detection of Class 1 Integron Among *Klebsiella* Species Isolated from Clinical Samples at No (1) Defence Services General Hospital (1000-Bedded)

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ABSTRACT

Klebsiella species is commonly associated with serious nosocomial infections. Multi-drug resistant *Klebsiella* species isolates are becoming increasingly prevalent in the clinical and nosocomial environments. The high prevalence of *Klebsiella* infections is related to the ability of *Klebsiella* species to acquire and disseminate exogenous genes associated with mobile elements, such as plasmids, transposons and integrons. This study was conducted to find out the presence of class 1 integron and antibiotic susceptibility patterns of *Klebsiella* species from clinical specimens at No (1) Defence Services General Hospital (1000-Bedded). A laboratory based cross-sectional descriptive study was carried out from January to september, 2020. Identification and antimicrobial susceptibility testing of *Klebsiella* species was performed by VITEK 2 Compact Analyzer. Class 1 integrons were detected by conventional PCR. In this study, 110 *Klebsiella* species were isolated from various clinical specimens and most of the isolates were from medical ward (52.3%, 63 isolates). The highest rate of resistance was observed for ampicillin (100%) and Cefotaxime (97.3 %) whereas the lowest antibiotic resistance was to Amikacin (15.5%). Out of 110 *Klebsiella* isolates, 107 (97.3%) were multidrug resistant (MDR). Forty-four (40%) out of 110 *Klebsiella* isolates carried *int1* gene and all these isolates were MDR. However, there was no association between multidrug resistance and integron positivity (p value = 0.273). The presence of class I integron genes among *Klebsiella* species highlights the continued monitoring is necessary for prevention of wide dissemination of integrons and infections by MDR pathogens.

Keywords: *Klebsiella*, MDR, Class (1) integron, No. (1) DSGH (1000-Bedded)

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1 Introduction

Klebsiella species are gram-negative bacteria that are associated with nosocomial infections. *Klebsiella* species causes a wide range of infections, including pneumonias and bacteremia [1]. The worldwide development of multidrug resistant (MDR) strains of *Klebsiella pneumoniae* is a matter of great concern [2].

Dissemination of antibiotic resistance genes by horizontal transfer has led to the rapid emergence of antibiotic resistance among clinical isolates. The mobile genetic elements named integron**s**, contain genes for site-specific recombination systems that are capable of capturing and mobilizing the drug resistant genes. Among the different integron families, Class 1 integrons are found to be most prevalent in drug-resistant bacteria [3].

The presence of integrons among *Klebsiella* species might account for multiple-antibiotic resistance. A strong association between the presence of integrons and antimicrobial resistance has been established by recent studies in various regions of Asia, Europe and USA [4]. A study in Iran found that 46% (46/100) of *Klebsiella pneumoniae* isolates carried class I integron from different clinical specimens. In this study, significant association was observed between resistance to antibiotics and presence of class I integron (p value = 0.05) [5]. Among 181 *Klebsiella pneumoniae* isolates from clinical specimens in Kashan, Iran, 150 (82.9%) isolates were identified as MDR during 2013 to 2014 study period. Of the MDR *Klebsiella pneumoniae* isolates, 150 (100%) carried *intI* 1 gene [6].

Many studies have established a strong association between the presence of integrons and antimicrobial resistance. Although few studies have documented the prevalence of integrons in *Klebsiella* species isolated from clinical and hospital environment of our military institution, more information regarding the association between Class 1 integrons and MDR still needs to be studied. The present study aimed to detect the Class 1 integron and *Klebsiella* species isolated from clinical samples at No (1) Defence Services General Hospital (1000-Bedded).

2 Materials and Methods

A total of 2297 various clinical samples were received at Microbiology Laboratory during January to September 2020. Sputum samples having >25 polymorphonuclear cells (PMNs) /HPF (100x) and <10 squamous epithelial cells /HPF (100x) in Gram-stained smears and all the urine, wound swab, and endotracheal aspirates were inoculated in both Blood agar and Mac'Conkey agar. After incubation at 37℃ aerobically for 18-24 hours, incubated plates were examined. Identification and antimicrobial susceptibility testing of isolated organisms were assessed by VITEK 2 Compact Automated Microbiology Analyzer. Non-dublicated 110 *Klebsiella* species isolate were stored in Trypticase soy broth with 20% glycerol at -20℃ until molecular study.

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. 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% agarose gels and were visualized under UV light.

3 Results

Among 2297 clinical samples were received at Microbiology Laboratory, 110 (28.5%) *Klebsiella* species was obtained. The highest yield of *Klebsiella* species (15.26%, 58 of 380) was observed from sputum followed by wound swabs (6.9%, 15 of 218), urine (3.38%, 21 of 620), other samples (1.75%, 9 of 515) and blood (1.24%, 7 of 564) (Figure 1).

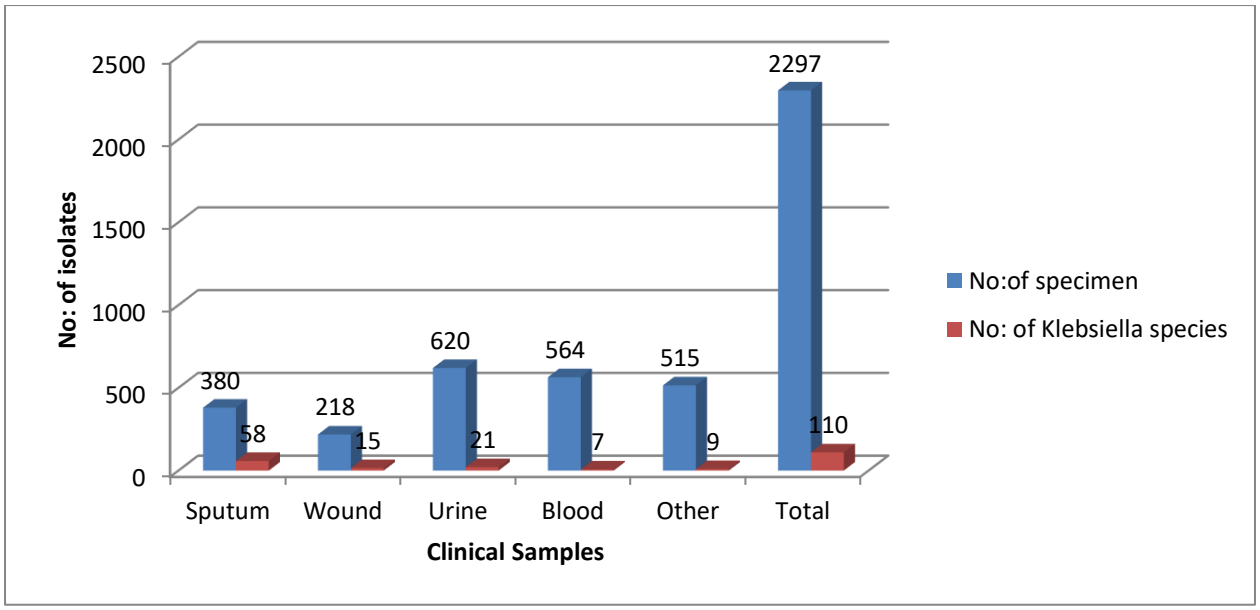

Figure 1: *Distribution of Klebsiella species isolated from various clinical specimens*

Among 110 *Klebsiella* species isolates, 63 (52.3%) isolates were from medical ward, 43 (39.1%) from surgical ward and 4 (3.6%) from ICU (Table 1).

Table 1: *Distribution of Klebsiella species isolates in different hospital wards*

Wards	**No: of Isolated *Klebsiella* species (%)**
Medical	63 (52.5%)
Surgical	43 (39.1%)
ICU	4 (3.6%)
Total	110 (100%)

The highest resistant rate was observed against ampicillin (100%), Cefotaxime (97.3%), Ceftriaxone (96.4%), Aztreonam (95.5%), Cefazolin (95.5%), Cefepime (94.5%), Ampicillin/Sulbactam (84.5%) and Nitrofurantoin (82.7%). Resistance to Ciprofloxacin, Levofloxacin, Tetracycline, Trimethoprim/Sulphamethoxazole, Gentamicin, Piperacillin/Tazobactam and Amoxicillin/Clavulanic acid were (79.2%), (77.3%), (75.5%), (75.5%), (61%), (55.5%), (54.5%) respectively. While the isolated *Klebsiella* species were susceptible to Amikacin (84.5%), Ertapenem (77.3%) and Imipenem (70%). (Figure 2).

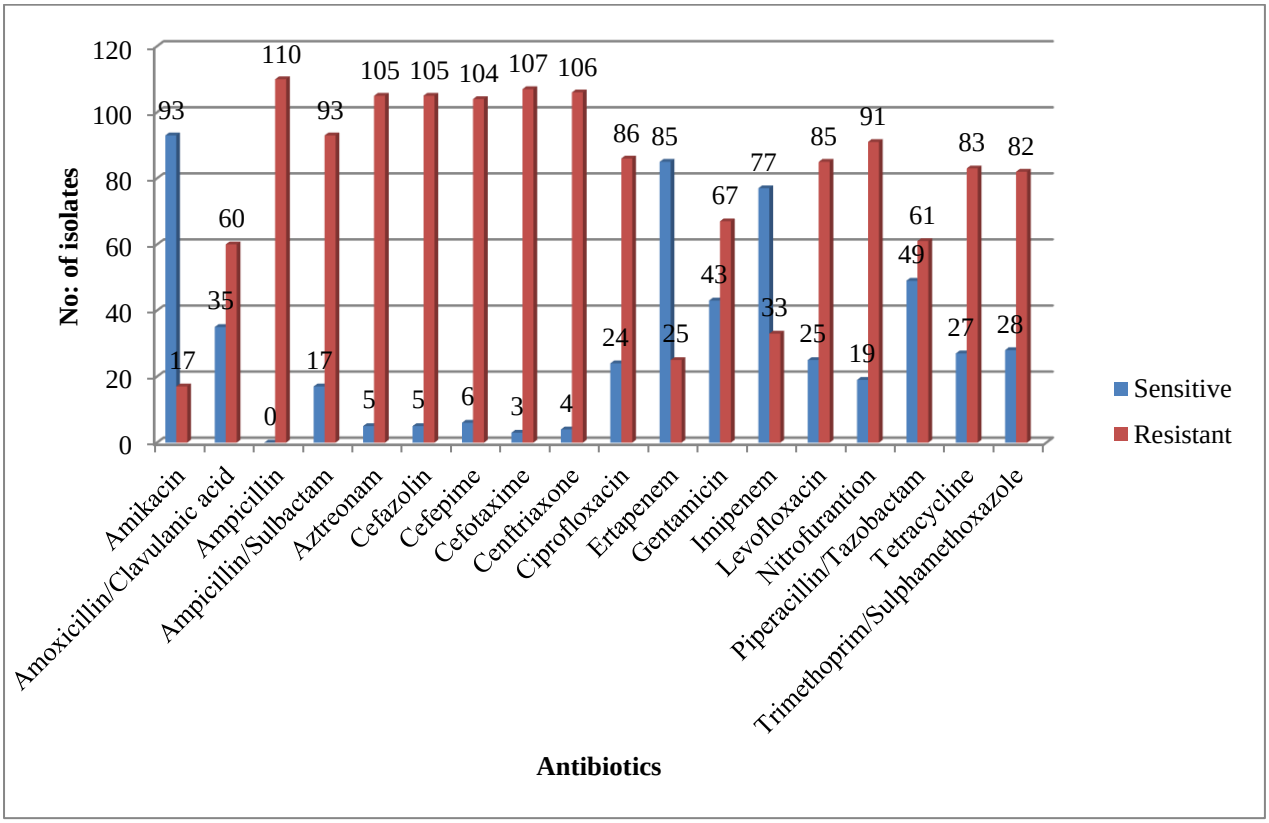

Figure 2: *Antimicrobial susceptibility pattern of the isolated Klebsiella species (N=110)*

Among 110 *Klebsiella* species isolates, 107 (97.3%) isolates were MDR strains. Forty percent (44 out of 110) *Klebsiella* isolates carried integron *int1* gene and all these isolates were MDR. There was no association between multidrug resistance and integron positivity, p value (p=0.273) in the present study (Table 2).

Table 2: *Association between multidrug resistant (MDR) Klebsiella species and class 1 integron positivity*

MDR	**Class (1) Integron positive (%)**	**Class (1) Integron negative (%)**	**Total**	***P* value**
Positive	44(41.1%)	63(58.9%)	107(100%)	0.273
Negative	0(0%)	3(100%)	3(100%)	
Total	44(40%)	66(60%)	110(100%)	

In this study, there were significant association between integron positivity and resistant to amoxicillin/clavulanic acid (p=0.037), ciprofloxacin (p=0.030), levofloxacin (p=0.020), nitrofurantoin (p=0.021), piperacillin/tazobactam (p=0.028) and trimethoprim/ sulphamethoxazole (p=0.020) (Table 3).

Table 3*: Association between antibiotic resistance and class (1) integron positivity among isolated Klebsiella species*

Antibiotic group	**Tested Antibiotics**	**Integron positive**		**p value**
		% R (no)	**% S (no)**	
Penicillin	Amc/Clv	46.7% (35)	25.7% (9)	**0.037**
	Ampicillin	40% (44)	-	
	Ampicillin/Sulbactam	44.1% (41)	17.6% (3)	**0.058**
	Piperacillin/ Tazobactam	49.2% (30)	28.6% (14)	**0.028**
Cephalosporin	Cefazolin	41.0% (43)	20% (1)	0.646
	Cefepime	41.3% (43)	16.7% (1)	0.399
	Cenftriaxone	41.5% (44)	0% (0)	0.148
Carbapenem	Aztreonem	41.0% (43)	1 (20%)	0.646
	Ertapenem,	28.0% (7)	43.5% (37)	0.164
	Imipenem	36.4% (12)	41.6% (32)	0.610
	Meropenem,	33.3% (12)	43.2% (32)	0.320
Aminoglycoside	Amikacin	47.1% (8)	38.7% (36)	0.518
	Gentamycin	44.8% (30)	32.6% (14)	0.202
Fluoroquinolone	Levofloxacin	45.9% (39)	20.8% (5)	**0.020**
	Ciprofloxacin	45.3% (39)	20.8% (5)	**0.030**
Tetracycline	Tetracycline	43.4% (36)	29.6% (8)	0.205
Nitrofurantoin	Nitrofurantoin	45.1% (41)	15.8% (3)	**0.021**
Sulphonamide	Trimethoprim/ Sulphamethoxazole	46.3% (38)	21.4% (6)	**0.020**

4 Discussion

Among 2297 clinical samples were received at Microbiology Laboratory, 110 (28.5%) *Klebsiella* species was obtained. Culture positivity of *Klebsiella* species from present study was also in agreement with 2018 (27% = 148 out of 541 isolated bacterial pathogens) and 2019

(31%= 231 out of 746 gram-negative bacterial isolates) data from No (1) DSGH (1000-bedded). Culture positivity of *Klebsiella pneumoniae* from various clinical samples of hospitalized patients in a tertiary care hospital of North India was 9% (194 of 2155 isolates) and Dhaka Medical College Hospital was 23.73% (75 of 316) [7].

The variation in frequency of isolation rate of *Klebsiella* species may be due to variation in the study population, geographical regions, types of samples, sample size, and different in detection methods during study period. The present study was conducted in only one tertiary care teaching hospital so the situation may differ in other parts of the countries.

The highest yield of *Klebsiella* species (15.26%, 58 of 380) was observed from sputum followed by wound swabs (6.9%, 15 of 218), urine (3.38%, 21 of 620), other samples (1.75%, 9 of 515) and blood (1.24%, 7 of 564) (Figure 1). The isolation rate of *Klebsiella* species was highest from sputum followed by wound swabs were similar with the findings of other studies. Majority of the *Klebsiella* species were isolated from the sputum (45.8 %) and wound swabs (34.5 %) in a Saudi hospital [8].

In present study, the highest number of *Klebsiella* species was identified from medical ward (52.3% = 63 of 110 isolates) followed by surgical ward (39.1%, 43 of 110 isolates) and 3.6% (4 isolates) from ICU (Table 1). The high incidences of *Klebsiella* infections could correlate with a decreasing immune system function. Patients with diabetes or malignancy more susceptible to get *Klebsiella* infection. Use of an invasive medical instrument for a long time contribute to incidences of *Klebsiella* infections.

Among tested antibiotics in the present study (Figure 2), 100% isolates showed resistance to **Ampicillin**. The cause may be chromosomally encoded β-lactamases responsible for intrinsic resistance. This was in agreement with the study of Ghanem et al, from Saudi, Gill et al, from North India [8][2]. In the present study, a high level of resistant is observed to **Cephalosporins** like Cefotaxime, Ceftriaxone Cefepime and Cefazolin. This was in agreement with the results of Gill et al., 2019 and Sonia et al., 2020 [2][7]. High resistance rate to Cephalosporins might be due to uncontrolled consumption of these antibiotics.

The resistance to **flouroquinolones** could be due to mutations in the chromosomal genes encoding DNA gyrase of the bacteria or due to efflux of the drug. A high level of resistant is also observed to the **fluoroquinolones** in which resistance to Ciprofloxacin, and Levofloxacin were (77.3%) and (75.5%) respectively. Studies performed in North India and Bangladesh where resistance to Ciprofloxacin was recorded 96% and 88% respectively which was higher than finding from present study [2][7]. Indiscriminate use of flouroquinolones against bacterial infections is mostly responsible for the emergence of these resistant isolates. In the present study, 75.5%% of *Klebsiella* species were resistance against sulphamethoxazole-trimethoprim, which is lower than Bangladesh findings (90.67% resistance) [7].

Klebsiella species showed moderate level of resistance (55.5%) against **Piperacillin-Tazobactam**. Studies performed by Gill et al and Sonia et al reported 89 % and 72% resistant to Piperacillin-Tazobactam respectively [2][7]. **Amikacin** is a fourth-generation aminoglycoside which showed good sensitivity (84.5%) in the present study. These results being consistent with North India study [2]. Among **Carbapenems,** susceptible to Ertapenem was (70%), and Imipenem was (67.27%) in the present study. It has been documented that enzyme KPC carbapenemase confers resistance to carbapenems, penicillins, and extended

spectrum cephalosporins as well. A high level of carbapenem resistance has also been reported in Ethiopia, 2020 [9]. In which from *Klebsiella* isolates from Ethiopia were highly sensitive to Ertapenem (91.8%) and Meropenem (87.8%).

In the present study, 97.3 % (107 of 110) of *Klebsiella* species isolates were MDR strains (Table 2). These are now being recognized as one of the major threats for effective management of patients in hospital. The drastic rise in the incidence of MDR *Klebsiella* species in No (1) DSGH (1000-bedded) when compared with hospital data. The occurrence of multidrug resistant *Klebsiella* species in present study was significantly higher than other studies. Among, 122 *Klebsiella pneumoniae* isolates from hospital at Klaten, Indonesia, MDR were (57.28%) [10].

The *Int1*gene has been reported as the most common and widespread, especially in clinical settings. In the present study, 40% (44 out of 110 isolates) *Klebsiella* species carried integron *int1* gene and all these isolates were MDR (Table 2). Findings from present study were in contrast to the Shakib study, in which the *intI1* gene was detected in 18.5% (13 of 70) of *Klebsiella pneumoniae* isolates from Hospitals of Sanandaj, Iran. Out of 28 multidrug resistant isolates, 11 isolates (39 %) were identified to be positive for the existence of class 1 integrons [11].

In this study, a significant association between integron carriage and higher rates of resistance to amoxicillin/clavulanic acid, ampicillin/sulbactam, ciprofloxacin, levofloxacin, nitrofurantoin, piperacillin/ tazobactam and trimethoprim/sulphamethoxazole ($p<0.05$) as shown in (Table 3). Class 1 integrons were detected in 18 out of 21 (85.7%) multidrug-resistant *Klebsiella* species isolated from Outpatients in Yazd, Iran. A significant relationship was found between integron and multidrug resistant phenotype [12].

Interestingly, there was no association between multidrug resistance and integron positivity, p value ($p=0.273$) in the present study as shown in (Table 2). This finding was concordance with study conducted in Iran by Mobarak-Qamsari. Mobarak-Qamsari et al found that there was no association between the presence of the integrons and MDR *Klebsiella pneumoniae*. However, in the integron positive isolates, the frequency of resistance to seven antibiotics from five classes was significantly higher than integron negative strains ($p < 0.05$) [13].

5 Conclusion

The prevailing trend of the co-occurrence of class 1 integrons and antimicrobial multiresistance is an additional threat for the spread of the antimicrobial multiresistance, which may further complicate future strategies for empirical therapy. Therefore, careful monitoring is necessary for the prevention of wide dissemination of integrons and an increase in infections by MDR pathogens.

6 Declarations

6.1 Ethical Consideration

Ethical clearance was obtained by Institutional review Board of DSMA

6.2 Acknowledgement

We gave our special thanks to all personal 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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