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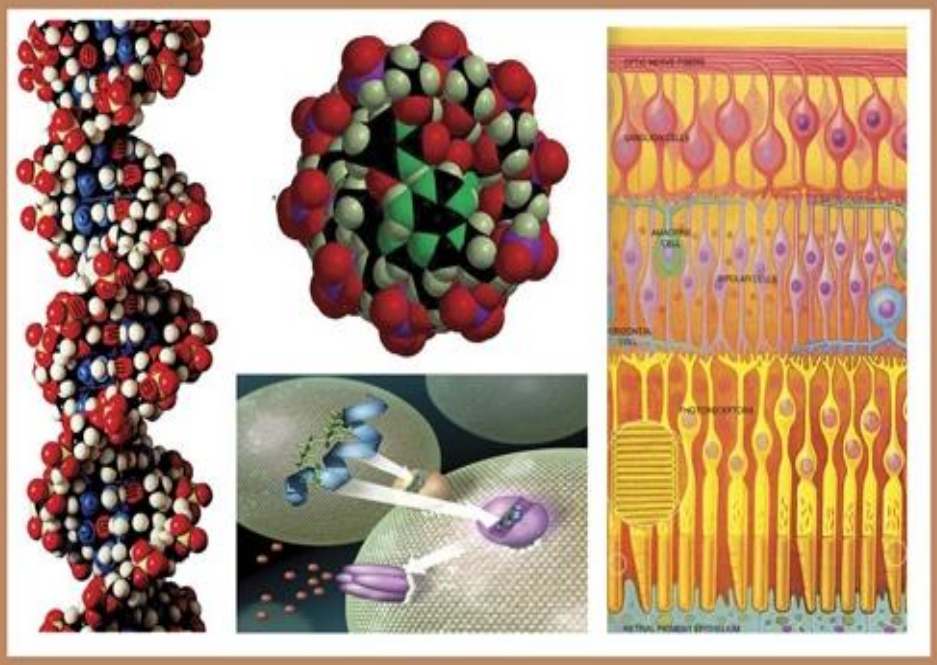

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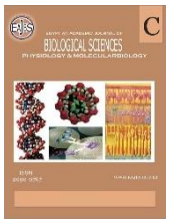

The Effect of Ceftriaxon and Gentamicin on Antibiotic Resistance Pattern of Multiple Drug Resistance *Klebsiella pneumoniae*

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ABSTRACT

Klebsiella pneumoniae is one of the bacterial species with high antibiotic resistance. The present study aimed to investigate the effect of Minimal Inhibitory Concentration (MIC) of Ceftriaxon and Gentamicin on the antibiotic pattern of multi-drug resistant *K. pneumoniae* isolated from urinary tract infections. Sixty *K. pneumoniae* isolates have been isolated from 300 urine specimens collected from patients with urinary tract infections. Vitek 2 Compact System was used for identification and detection of antibiotic resistance patterns of bacterial isoltes. The highest percentage of antibiotic resistance of bacterial isolates to ampicillin (100%), followed by cefazolin (53.3%), while the lowest percentage of resistance was shown to Imipenem and tigecycline, which were 5% and 3.3%, respectively. The effect of MIC of gentamicin and ceftriaxon toward 10 selected isolates were used to select mutated isolates, then the effect of both antibiotic-on-antibiotic resistance of mutated isolates was detected using Vitek 2 Compact System. The results of the mutation experiment using ceftriaxone gave successful results to 7 isolates while 5 isolates gave positive results to gentamicin. The results of antibiotic resistance pattern of mutated isolates showed that all mutated isolates became resistance to most tested antibiotics in comparison with original isolates. We concluded that indiscriminate and wrong use of antibiotics would lead to emergence of MDR isolates not only for antibiotics belonging to the same class but also to other classes.

INTRODUCTION

Klebsiella pneumoniae is one of the most common opportunistic pathogens associated with nosocomial infections, wound and urinary tract infections, pneumonia, bacteremia, meningitis, and liver abscesses (Tawfick *et al.*, 2016; Martin and Bachman, 2018).It showed high antibiotic resistance due to the possession of several resistance mechanisms in addition to the changes that occur in the bacterial genome of this bacteria (Ashurst and Dawson, 2020). The antibiotic resistance of bacterial cells is classified into (i) natural resistance represented by structural and functional characteristics (Kadhum *et al.*, 2019) and (ii) acquired resistance that occurs as a result of a genetic mutation and the acquisition of antibiotic resistance genes from other bacterial strains by horizontal genes transfer (Andersson *et al.*, 2021).

K. pneumoniae have several mechanisms of antibiotic resistance including (i) production of antibiotic-inhibiting enzymes that degrade antibiotics (ii) alteration of the target site of antibiotic action (iii) alteration of cell membrane permeability (iv) modification of metabolic pathways (v). In addition to having efflux pump systems, bacteria possess some of these mechanisms naturally and some acquire them by acquiring resistance genes (Verma *et al.*, 2015; Sawa *et al.*, 2020).

Genetic mutations are one of the most important mechanisms used by bacteria to develop antibiotic resistance, which appear in multiple sites on the bacterial chromosome or by acquiring resistance genes carried on the plasmid or transposon through horizontal gene transfer (Hammami *et al.,* 2011).

One of the most important problems facing hospitals is the significant rise in multiple-antibiotic-resistant *K. pneumoniae* (MDR) and extensively antibiotic-resistant (XDR) strains, which represent a threat to public health. The increase in the emergence of Crbapenem Resistance Enterobacterales (CRE) strains has become a serious threat to public health, where such strains can cause high mortality due to the difficulty of treatment (Suay and Perez, 2019), and such strains that produce Carbapenemase enzyme have a high level of resistance to other antibiotics. The increase in the rate of antibiotic resistance and the lack development of new antimicrobial drugs led to the spread and development of bacterial infections (Pacios *et al.,* 2020). So, this research aimed to investigate the effect of MIC of ceftriaxone and gentamicin on antibiotic resistance pattern of *K. pneumoniae*.

MATERIALS AND METHODS

Isolation and Identification of *K. Pneumoniae*:

Three hundred urine specimens were collected from patients with UTI, cultured directly on MacConkey agar and incubated for 24hr. at 37°C for primary isolation of *K. pneumoniae*. Pure suspected colonies were further identified using Vitek 2 Compact System (ID/GN).

Antibiotic Resistance Test:

Vitek 2 Compact System for antibiotic sensitivity test (AST) was used for detection of antibiotic susceptibility of all *K. pneumoniae* isolates.

Study the Effect of Ceftriaxone and Gentamicin on MDR *K. pneumoniae:*

Method described previously (Lentino *et. al.,* 1993) was followed up to evaluate the effect of MIC of ceftriaxone and gentamicin on antibiotic resistance pattern of 10 selected isolates of MDR *K. pneumoniae*. Briefly, young bacterial isolates of *K. pneumoniae* (1ml) have been transferred to inoculate Mueller Hinton Broth (MHB) supplemented with 0.5µg/ml and 16 µg/ml of gentamicin separately. Also, young bacterial isolates (1ml) have been transferred to inoculate MHB supplemented with 0.5µg/ml, 32 µg/ml and 64 µg/ml of ceftriaxone separately. All cultures' tubes were incubated at 37°C for 24hr. The appearance of bacterial growth referred to the presence of mutated colonies. Then, 100µl of bacterial culture was spread on MHA free from antibiotics and incubated at 37°C for 24hr. To select irreversible mutant colonies, at the end of incubation period, a loopful of bacterial colonies were selected randomly and incubated on MHA supplemented with the same concentration of gentamicin and ceftriaxone and incubated at 37°C for 24hr. All colonies that grew on MHA supplement with antibiotics represented mutated colonies.

Detect Antibiotic Resistant Pattern of Mutated Isolates:

Vitek 2 Compact System for antibiotic sensitivity test (AST) was used for detection of antibiotic susceptibility of all gentamicin and ceftriaxone mutated isolates of *K. pneumoniae*.

RESULTS

The results of Vitek 2 Compact System for identification of *K. pneumoniae* showed that out of 210 pure bacterial isolates that grow on MacConkey agar only 60 bacterial isolates belong to *K. pneumoniae* (Table 1).

The results of Vitek 2 Compact System to investigate the antibiotic resistance pattern of bacterial isolates (Table2) showed that a high percentage of resistance to ampicillin (100%) followed by cefazolin which was 53.3%, while the percentage of resistance to ceftazidime, ceftriaxone and cefepime was 45% for each of them. The percentage of resistance trimettoprim / sulfamethoxazol was 28.3%, and it was 21.66% for cefoxitin the percentage of resistance to. The results showed that the percentage of resistance to ciprofloxacin, levofloxacin and gentamicin

was 11.6% each. The lowest percentage of resistance was to pipracillin / tazobactam, imipenem and tigecycline was 8.3%, 5% and 3.3%, respectively, while the percentage of resistance to Nitrofurantin, amikacin and ertapenem was 6.66% for each of them. Multiple antibiotic resistant (MDR) and extensively resistant to antibiotics (XDR) *K. pneumoniae* was reported in this study.

Table 1: Biochemical tests for the diagnosis of *K. Pneumoniae* using the Vitek 2 Compact System.

AI_AMAL Lab
Laboratory Report

bioMérieux Customer:
System #:
Patient Name: Mac. 1 Pet., Adian
Isolate: 7120231-1 (Approved)
Card Type: GN Bar Code: 241180710337187 Testing Instrument: 000014EEE4E4 (9513)
Setup Technologist: Laboratory Administrator(Labadmin)
Bionumber: 6607734753564210
Organism Quantity:

Printed by: System
Patient ID: 7120231

Selected Organism: Klebsiella pneumoniae ssp pneumoniae

Comments:	

Identification Information	**Card:** GN	**Lot Number:** 2411807103	**Expires:** Nov 3, 2023 12:00 CST
	Status: Final	**Analysis Time:** 3.85 hours	**Completed:** Jan 8, 2023 01:17 CST
Organism Origin	VITEK 2		
Selected Organism	99% Probability **Klebsiella pneumoniae ssp pneumoniae**		
	Bionumber: 6607734753564210	Confidence: Excellent identification	
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Table 2: The Percentage of Antibiotic Resistance of *K. pneumoniae* Isolates.

Antibiotics Class	Antibiotic (Symbol)	NO (%) of Bacterial Isolates Which is:		
		Sensitive	Intermediate	Resistant
Aminoglycoside	Amikacin (AK)	54(90)	2 (3.3)	4 (6.7)
	Gentamicin (CN)	53 (88.3)	–	7 (11.7)
Cephalosporin	Cefazolin	27 (45)	1 (1.7)	32 (53.3)
	Cefoxitin(FOX)	47 (78.3)	–	13 (21.7)
	Ceftazidime(CAZ)	33 (55)	–	27 (45)
	Ceftriaxon(CRO)	33 (55)	–	27 (45)
	Cefepime(CPM)	33 (55)	–	27 (45)
	Pipracillin / Tazobactam (PRL/TZ)	53 (88.3)	2 (3.3)	5(8.3)
Carbapenems	Imipenem (IMI)	55 (91.7)	2 (3.3)	3 (5)
	Erapenem	56 (93.3)	–	4 (6.7)
Aminopenicillins	Ampicillin(AM)	–	–	60 (100)
Fluroquinolones	Ciprofloxacin(CIP)	53 (88.3)	–	7 (11.7)
	Levofloxacin(LEV)	52 (86.6)	1(1.7)	7 (11.7)
Tetracyclines	Tigecycline(TE)	58 (96.7)	–	2 (3.3)
	Nitrofurantone(NTI)	35 (58.3)	21 (35)	4 (6.7)
Diaminopyrimidines	Trimethoprime(TMP)/ Sulfamethoxazole	43 (71.7)	–	17 (28.3)

Five isolates of *K. pneumoniae* showed the ability to grow in the presence of MIC of gentamicin while 7 isolates of *K. pneumoniae* showed the ability to grow in the presence of MIC of ceftriaxone which referred to the emergence of mutated isolates. The effect of MIC of gentamicin and ceftriaxone on antibiotic resistance pattern of mutated isolates, Vitek 2 Compact System has been carried out to investigate antibiotic resistance pattern of mutated isolates. As mentioned in Table 3, the results showed that all mutated isolated by both antibiotics developed resistance to most studied antibiotics where a high development of resistant observed in mutated isolates by ceftriaxone where it showed high antibiotic resistance to most studied antibiotics.

Table 3: Antibiotic resistant pattern of origin and mutated isolates by gentamicin and ceftriaxone**.**

NO. of Isolate	Antibiotic-Resistant Pattern of Origin Isolates	Antibiotic-Resistant Pattern of Mutated Isolates by Gentamicin	Antibiotic-Resistant Pattern of Mutated Isolates by Ceftriaxone
1	AM, CEF, CAZ, CRO, CPM, TMP/SUL, ESßL	AM, PRL/TZ, CEF, CAZ, CRO, CPM, CN, NTI, TMP/SUL, ESßL	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, AK, CN, CIP, LEV, TE, NTI, TMP/SUL
2	AM, PRL/TZ, CEF, CAZ, CRO, CPM, CN, ESßL	AM, CEF, CAZ, CRO, CPM, NTI, TMP/SUL, ESßL	AM, PRL/TZ,CEF,FOX, CAZ, CRO, CPM, ERA, IMI, CN, CIP, LEV, TE,NTI, TMP/SUL
3	AM, CEF,CAZ,CRO, TMP/SUL, ESßL	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, AK, CN, CIP, LEV, TE, NTI, TMP/SUL	AM, PRL/TZ, CEF, CAZ, CRO, CPM, IMI, LEV, TMP/SUL, ESßL
4	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, AK, CN, CIP, LEV, TE, NTI, TMP/SUL	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, CN, CIP, LEV, TE, NTI, TMP/SUL	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, AK, CN, CIP, LEV, TE, NTI, TMP/SUL
5	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, AK, CN, CIP, LEV, TE, NTI, TMP/SUL,	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, AK, CN, CIP, LEV, TE, NTI, TMP/SUL	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, AK, CN, CIP, LEV, NTI, TMP/SUL
6	AM, CEF,CAZ,CRO, CPM, ESßL	/	AM, CEF,CAZ,CRO, CPM, NTI, TMP/SUL, ESßL
7	AM, CEF, FOX,	/	AM, PRL/TZ, CEF, FOX, CAZ, CRO, CPM, ERA, IMI, AK, CN, CIP, LEV,TE, NTI,TMP/SUL

DISCUSSION

Urinary tract infection (UTI) is one of the common health problems in both sexes and at different ages, and it includes infection of the kidneys, ureters, and bladder. *K. pneumoniae* is one of the most important causes of UTI after *E. coli* and it constitutes 32% of the total enterobacteriaceae pathogens (Kareem and Rasheed, 2011; Levinson, 2016). Distribution of *K. pneumoniae* may due to possession of such isolates to many virulence factors that increase their virulence and ability to cause infection such as capsules that play an important role in resistant of bacterial isolates to phagocytosis (Kerstman *et al.*, 2012).

K. pneumoniae is one of the opportunistic pathogens with multiple antibiotic resistance, and belongs to the multidrug-resistant ESKAPE family, including *Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, *and Enterobacter* spp. (Mulani *et al.*, 2019).

The results of this study improved that exposure of bacterial isolates to antibiotic in MIC dose lead to a development of resistance to many classes of antibiotics and lead to emergence of mutants which developed many mechanisms to resist the action of antibiotics. The antibiotic resistance of bacterial isolates to ampicillin may be attributed to their ability to produce metallo-beta-lactamases and broad-spectrum beta-lactamases that degrade the antibiotic while its resistance to carbapenems, represented by Imipenem and Ertapenem, due to production of carbapenemase encoded by a plasmid-borne gene that was first detected in *K. Pneumoniae* isolates (Karlsson *et al.*, 2019).

Bacterial isolates have three mechanisms of resistance to tigecycline which are (i) their ability to chemically modify the antibiotic molecule, which prevents it from binding to the target site, and (ii) ribosomal protection, as bacterial isolates have the ability to produce Tet protein that works to protect ribosomes (the target site for action). (Brooks *et al.*, 2010) and (iii) the bacterial isolates possess efflux pumps that flush the antibiotic out of the bacterial cell, which is represented one of the most important mechanisms of resistance to these antibiotics.

The high resistance of *K. Pneumoniae* isolates towards aminoglycosides may be attributed to their possession of enzymes that inhibit the action of these antibiotics (Jabar and Hasson., 2019).A low resistance was shown to diaminopyrimidines, represented by trimethoprim/sulfamethoxazole, which may due to different mechanisms including their ability to replace some steps in the metabolic pathway or inhibit the permeability of biofilm or production of dihydrofolate reductase (DHFR) that encodes by chromosome or plasmid which increase resistance to the trimethoprim (Capasso and Supuran, 2019). Fluoroquinolones resistant was mediated by efflux pumps that reduce the permeability of the antibiotic (Yedekci *et al.,* 2012). The results of the current study confirmed that a high percentage of MDR and XDR *K. pneumoniae* was detected which may due to the ability of bacterial cells to acquisition of resistance genes that transmitted horizontally or vertically where the horizontal transfer of genes is the most effective and dangerous, as it works on spreading genes easily between different strains, either through plasmids or transposons (Oladeinde *et al.,* 2022).

CONCLUSION

K. pneumoniae represented one of the most causes of UTI and prevalence of MDR *K. pneumoniae.* Exposure of bacterial isolates to MIC of antibiotics develops resistant to most classes of antibiotics which in turn results in difficult in treatment of such infections.

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