Anti–biotypes of Different Bacteria Isolated from Different Clinical Sources

Rebwar M. Hama Salih , Khadija K. Mustafa , Zirak F.A. Abdulrahman
Department of Biology, College of Education, Salahaddin University , Erbil, Iraq

Abstract
A collective of 1178 different isolates were collected and identified from clinical specimens of human patients and these sources include urine (n=641), swab (n=483), wound (n=29) and blood (n=25). A present study were confirmed on antibiotic resistance against some pathogenic bacterial genera (n=1178) which include *Escherichia coli* (n=417), *Staphylococcus aureus* (n=377), *Klebsiella pneumoniae* (n=212), *Pseudomonas aeruginosa* (n=145) and *Streptococcus* spp. (n=27) isolates; was carried out in Internal Lab from Teaching Hospital in Erbil city, in September 15, 2012 to June 20, 2013. Susceptibility was determined by the disc diffusion method recommended by the Clinical and Laboratory Standard Institute (CLSI). The following antimicrobials were tested: AMC 20µg, AK (30µg), AM (30µg), AZM (15µg), ATM (30µg), CAR (100µg), CF (30µg), CEC (30µg), CFZ (15µg), CD (5µg), CFM (30µg), CPO (30µg), CP (75µg), FOX (30µg), CPR (30µg), CXM (30µg), KF (30µg), C (30µg), CN (100µg), CIP (5µg), CLM (15µg), DA (2µg), CT (10µg), E (15µg), G (10µg), GIP (5µg), IPM (10µg), DP (5µg), NAF (1µg), NF (30µg), F (300µg), NOR (10µg), OX (1µg), PG (10µg), PIP (100µg), RA (5µg), TE (30µg), TC (75µg), TOB (10µg), SXT (1.25/23.75µg), and VA (30µg). The resistances percent of all bacterial isolates show different range of resistant which start from 0.00% and reach to 100%.

Keywords: Disc diffusion of Antibiotic Susceptibility, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *Streptococcus* spp.

Introduction
Antibiotics are specific chemical compounds derived from or produced by microorganisms that even in small amounts can selectively inhibit the growth of the life processes or growth of other microorganisms [1]. Antibiotic resistance and reduction in the effectiveness of antibiotics to treat certain bacterial infections in humans has been a growing concern internationally. There has always been consensus that the development of resistance is associated with continued use of antibiotics [2]. Antimicrobial drug resistance is one of the major threats due to widespread use of antimicrobial drugs in general population. Also, it is known that the common infecting organism and pattern of resistance changes over time (changing trends). It can arise from the selection of resistant strains among naturally susceptible species or from the ingress of new strains of naturally resistant species. The extent of use of particular agents in a given environment dictates the rate at which resistance arises among microbial populations [3].

E. coli is the most common cause of infections by gram negative bacilli and it is a frequent cause of outpatient urinary tract infections in women worldwide, septicemia, diarrhea and meningitis. Resistance to recommended first and second line agents, such as penicillin, cephalosporin, sulfa drugs and fluoroquinolones [4, 5, 6, 7].

S. aureus has emerged as one of the most important human pathogens and has over the past several decades, been a leading cause of hospital and community acquired infections. One of the reasons for the success of this human pathogen is its great variability, occurring at different periods and places with diverse clonal types and antibiotic resistance patterns within regions and countries. Although infections caused by antibiotic resistant *S. aureus* bring about serious problems in the general population, such infections can be particularly devastating for the very young, the elderly and the immunocompromised [8,9]. Development of resistance to antimicrobial agents by *S. aureus* is a major concern primarily because they are still frequently associated with hospital and community acquired infections. The organisms exhibit remarkable versatility in their behavior towards antibiotics, with some strains having overcome most commonly used drugs. Exposure to new antibiotics often results in further selection of homologous resistant strains, a phenomenon particularly favored by irrational antibiotic administration [2].

K. pneumonia is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae [10]. *K. pneumonia* is resistant to a number of antibiotics mainly extended spectrum cephalosporin's and penicillin's due to acquisition of plasmid that encode for the production of extended spectrum beta lactamases (ESBL) especially TEM and SHV enzymes have been described worldwide [11].

P. aeruginosa is commonly associated with hospital acquired infection, most notably in immunocompromised individuals [12,13] and accounts for 10% of all hospital acquired infections. Specifically, the second most frequently recovered pathogen from intensive care unit (ICU) patients, those with neutropenia (low white blood cell count) [14]. *P. aeruginosa* is intrinsically resistant to narrow spectrum penicillins, first and second generation cephalosporins, trimethoprim, and sulfonamides. The anti–pseudomonal agents include extended spectrum penicillins, such as Ticarcillin and Pipercillin; extended spectrum Cephalosporin's, such as Ceftazidime and Cefepime; Carbapenem; aminoglycosides; and fluoroquinolones [15].

Streptococcus is a very heterogeneous group of bacteria; some members are a part of human normal flora while others are potent pathogens. The primary pathogens are *S. pyogenes* and *S. pneumoniae* but

other species can be opportunistic. For example, *S. agalactiae* can produce severe neonatal disease including meningitis, pneumonia and bacteremia in infants. *S. mutans* is an important contributor to dental caries. Nonpneumococcal streptococci is classified into two groups according to their ability to hemolyze sheep red blood cells. Those isolates that completely lyse or hemolyze red blood cells are called beta-hemolytic streptococci. Based upon antigenic characteristics of the C carbohydrate located in their cell wall the β–hemolytic streptococci are further classified into groups A, B, C, D, F and G**.** Those species that only partially hemolyze red blood cells are called viridans group streptococci. There are at least 20 species of viridans streptococci. The viridans streptococci are members of the normal flora of the gastrointestinal and respiratory tracts of humans [16].

Materials and Methods

Specimen's collection; a total of 1178 isolates of bacteria which were *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *Streptococcus* spp collected and isolated from different clinical sources like; urine, swab, wound and blood taken from patients who admitted to Internal Lab of Teaching Hospital in Erbil city during the periods of September 15, 2012 to June 20, 2013. In addition to the biochemical tests and different culture media, different API identification system (bioMèrieux, France) was performed to support and complete the diagnosis and identification of all isolates.

Media, chemicals and reagents; all the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. and Oxoid Ltd. Media used in this study included: Nutrient, MacConkey, Blood, Mueller Hinton and Mannitol Salt agars. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 min at 15 p/ inch^2 pressure.

Isolation and identification of isolates; discrete colonies were subcultured into fresh agar plates aseptically to obtain pure cultures of the isolates. All isolates were gram stained to determine their gram reaction. Mannitol fermentation tests were carried out. Other tests carried out were Coagulase, Catalase, Urease activity, Oxidase, Gelatinase Solidification test, DNase test, Motility test, IMViC tests and Clumping factor test.

Inoculum preparation; Nutrient broth was prepared, and five discrete colonies of the different identified isolates were inoculated into 5 ml of the broth and incubated at 35–37 °C. A spectrophotometer was used to monitor the turbidity of the broth cultures. Immediately the turbidity exceeded the 0.5 McFarland standard solutions, the incubation was stopped. The broth culture was then diluted nutrient broth to give a count of approximately 1.5 * 10^8CFU/ml.

Susceptibility studies; the antibiotic susceptibility test was performed for all genera and isolates against 41 antibiotics which were Amoxiclave (AMC 20µg), Amikacin (AK 30µg), Amoxicillin (30µg), Azithromycin (AZM 15µg), Aztreonam ATM 30µg), Carbenicillin (CAR 100µg), Cefaclor (CF 30µg), Cefotaxime (CEC 30µg), Cefazolin (CFZ 15µg), Cefdinir (CD 5µg), Cefixime (CFM 30µg), Cefonicid (CPO 30µg), Cefoperazone (CP 75µg), Cefoxitin (FOX 30µg), Cefprozil (CPR 30µg), Cefuroxime (CXM 30µg), Cephalothin (KF 30µg), Chloramphenicol (C 30µg), Cinoxacin (CN 100µg), Ciprofloxacin (CIP 5µg), Clarithromycin (CLM 15µg), Clindamycin (DA 2µg), Colistin (CT 10µg), Erythromycin (E 15µg), Gentamycin (G 10µg), Grepafloxacin (GIP 5µg), Imipenem (IPM 10µg), Methicillin (DP 5µg), Nafcillin (NAF 1µg), Nalidixic acid (NF 30µg), Nitrofurantoin (F 300µg), Norfloxacin (NOR 10µg), Oxacillin (OX 1µg), Penicillin G (PG 10µg), Pipercillin (PIP 100µg), Rifampin (RA 5µg), Tetracycline (TE 30µg), Ticarcillin (TC 75µg), Tobramycin (TOB 10µg), Trimethoprim–Sulfamethoxazole (SXT 23.75µg), and Vancomycin (VA 30µg) as clarified in Table (1), and were determined according to National Committee for Clinical Laboratory Standards (**NCCLS**) [17].

Table (1) Antibiotics name, abbreviation (symbol), final concentration (as printed on discs), and zone inhibition diameters (mm)

Antibiotics	Symbol	Concentration (µg or U)	Inhibition Zone diameter (mm)		
			R**	I	S
Amoxiclave*	AMC*	20	≤ 19	…..	≥ 20
Amikacin	AK	30	≤ 14	15-16	≥ 17
Amoxicillin	AM	30	≤ 8	16	≥ 32
Azithromycin	AZM	15	≤ 2	3-7	≥ 8
Aztreonam	ATM	30	≤ 15	16-21	≥ 22
Carbenicillin	CAR	100	≤ 13	14-16	≥ 17
Cefaclor	CF	30	≤ 14	15-17	≥ 18
Cefotaxime	CEC	30	≤ 14	15-22	≥ 23
Cefazolin	CFZ	15	≤ 8	16	≥ 32
Cefdinir	CD	5	≤ 16	17-19	≥ 20
Cefixime	CFM	5	≤ 15	16-18	≥ 19
Cefonicid	CPO	30	≤ 14	15-17	≥ 18
Cefoperazone	CP	75	≤ 15	16-20	≥ 21
Cefoxitin	FOX	30	≤ 14	15-17	≥ 18
Cefprozil	CPR	30	≤ 14	15-17	≥ 18
Cefuroxime	CXM	30	≤ 25	26-30	≥ 31
Cephalothin	KF	30	≤ 14	15-17	≥ 18
Chloramphenicol	C	30	≤ 12	13-17	≥ 18
Cinoxacin	CN	100	≤ 16	32	≥ 64
Ciprofloxacin	CIP	5	≤ 15	16-20	≥ 21
Clarithromycin	CLM	15	≤ 13	14-17	≥ 18
Clindamycin	DA	2	≤ 14	15-20	≥ 21
Colistin	CT	10	≤ 10	…..	≥ 11
Erythromycin	E	15	≤ 13	14-22	≥ 23
Gentamycin	G	10	≤ 12	13-14	≥ 15
Grepafloxacin	GIP	5	≤ 14	15-17	≥ 18
Imipenem	IPM	10	≤ 13	14-15	≥ 16
Methicillin	DP	5	≤ 9	10-13	≥ 14
Nafcillin	NAF	1	≤ 10	11-12	≥ 13
Nalidixic acid	NA	30	≤ 25	…..	≥ 26
Nitrofurantoin	F	300	≤ 14	…..	≥ 17
Norfloxacin	NOR	10	≤ 12	13-16	≥ 17
Oxacillin	OX	1	≤ 10	11-12	≥ 13
Penicillin G	PG	10	≤ 28	…..	≥ 29
Pipercillin	PIP	100	≤ 17	…..	≥ 18
Rifampin	RA	5	≤ 16	17-18	≥ 20
Tetracycline	TE	30	≤ 11	12-14	≥ 15
Ticarcillin	TC	75	≤ 14	…..	≥ 15
Tobramycin	TOB	10	≤ 12	13-14	≥ 15
Trimethoprim-Sulfamethoxazole	SXT	23.75	≤ 10	11-15	≥ 16
Vancomycin	VA	30	≤ 14	15-16	≥ 17

Key *: Antibiotics, symbols, concentrations and resistance is defined according to Clinical and laboratory standards institute (CLSI); **: R: Resistant; I: Intermediate of Resistance; S: Sensitive.

Results and Discussion

Collection of bacterial genera isolates; collection of 417 isolates of *E. coli*, 377 isolates of *S. aureus*, 212 isolates of *K. pneumoniae*, 145 isolates of *P. aeruginosa* and 27 isolates of *Streptococcus* spp were performed, isolated and identified depending on morphological, cultural and biochemical tests including different system of API strips as shown in Table (2) and Figure (1) and (2).

Table (2) Results of Morphological Features, Cultural Characteristics and Biochemical Tests for All Bacteria Genera

Bacterial Genera	Colonies Feature on Culture Media	Biomedical Tests	Motility Test	Capsule Possessing	Endospore Forming Bacteria	The Profile Code Number in API System
E. coli	Dark center with greenish metallic sheen colonies on EMB agar (selective media), Red or shiny pink dry colonies with rapid lactose fermenting on MC	Indole positive, Methyl red positive, VP negative, Citrate utilization negative, Oxidase negative, Urease negative	Motile	Non – capsulated bacteria	None	5 144 552 5 144 572
K. pneumoniae	Large, mucoid, brownish on EMB Pink, large, glistening and mucoid colonies with rapid lactose fermenting on MC	Indole negative, Methyl red negative, VP positive, Citrate utilization positive, Gelatinase negative, Oxidase negative, Urease positive	Non – Motile	Capsulated bacteria	None	2 004 343 1 214 773 5 215 773
P. aeruginosa	Translucent, colorless to gold on EMB, Transparent, colorless on MC, Secrete pyocyanin pigment on nutrient agar and change the pale red color of medium to green color	Oxidase positive, Citrate utilization positive, Urease positive, DNase negative	Motile	Non – capsulated bacteria	None	2 200 026 2 206 004
S. aureus	Creamy/buff colored colonies surrounded by a zone of complete B hemolysis, Shiny yellow colonies and change the pink color of MSA to yellow	Catalase positive, VP positive, Coagulase positive, DNase positive, Gelatinase positive, Oxidase negative, Indole negative, Methyl red negative	Non – Motile	Non – capsulated bacteria	None	…….
***Streptococcus* spp**	Shown different types of hemolysis on Blood agar	Catalase negative	Non – Motile	Non – capsulated bacteria	None	0470000 92% 0472410 99% 5040710 98%

Figure (1) API Strip Results of Pathogenic Bacteria

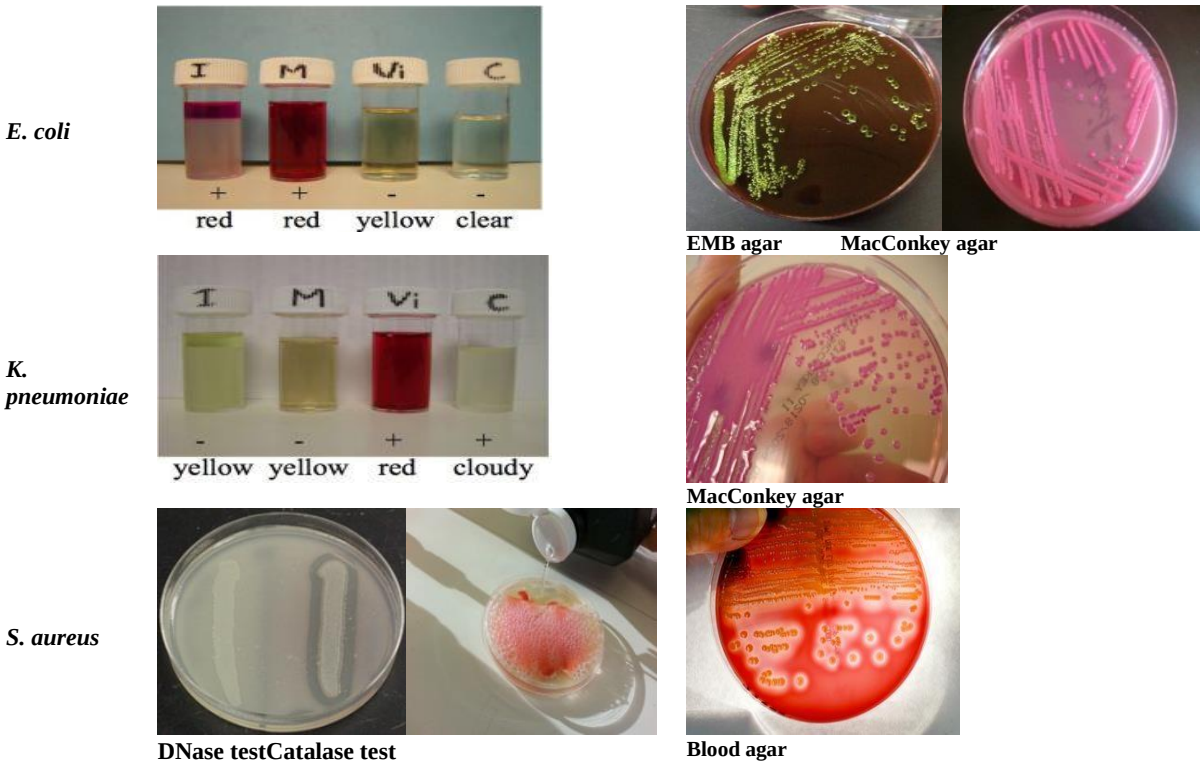

Figure (2) Biochemical Tests and Cultural Characteristics of Pathogenic Bacteria

Distribution and percent rates of the bacterial genera isolates according to their source of infection; compilation of 1178 isolates of bacterial genera isolates include; *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *Streptococcus* spp were carried out and classified according to their source of infection, Table (3) shown that urine isolates were the most frequent by forming 54.5 %, while swab formed 41.02 %, then each of wound, and blood were 2.24%, however the samples were taken irregularly, but were dependent on the patients who admitted into these hospital.

Table (3) Distribution and Percent Rates of All Bacterial Isolates among Specimens

Source of Isolation	No. of Samples	% of Sample Isolates	No. (%) of Isolates				
			E. coli	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*	*Streptococcus* spp
Urine	641	54.41	**350**(54.63)	**132**(20.61)	**106**(16.49)	**40**(6.185)	**13**(2.06)
Swab	483	41.001	**67**(13.698)	**204**(42.465)	**99**(20.547)	**99**(20.547)	**14**(2.739)
Wound	29	2.46	**0**(0.00)	**29**(100)	**0**(0.00)	**0**(0.00)	**0**(0.00)
Blood	25	2.12	**0**(0.00)	**12**(50)	**7**(25)	**6**(25)	**0**(0.00)
Total	**1178**	**100%**	**417** (35.393)	**377**(32.022)	**212**(17.977)	**145**(12.359)	**27**(2.247)

Resistance rates of antibiotics for bacterial genera; Table (4) showed the resistance percent of forty one antibiotics which tested against five genera of bacterial isolates. The resistance rates were occurred between 0.00% and 100.00%., which the high resistance percent record in AZM, CD, CPR, E, and VA against *E. coli*, AZM, NA, NOR, PIP, and TOB against *S. aureus*, OX against *K. pneumoniae*, AK, CEC, CD, and TE against *Streptococcus* spp were 100%, while the highest percent in *P. aeruginosa* 90.91% was recorded for SXT, while the lowest resistance percent was 0.00% recorded in CEC, KF, NOR, and TC for *E. coli*, CN, DP, OX, and PG, to *S. aureus*, CEC, CFM, CPO, CP, FOX, and DP to *K. pneumoniae*, CXM, KF, CN, CLM, CT, and E to *P. aeruginosa*, and AM, ATM, FOX, DA, NA, and TC in *Streptococcus* spp. The other antibiotics were shown different range of resistance to all bacterial isolates. Toroglu and Keskin [11] demonstrated that resistance rate of 22 isolates of *K. pneumoniae* which collected from urine, vaginal fluid, wound, cerebrospinal fluid and blood against eleven antibiotics were 95% to PG, 82% to AM, 77% to CFZ, 59% to CPR and TC, 46% to G, 332% to F, 27% to FOX and OF, 23% to ST and 19% to C. Desai and Malek [18] used eight antibiotics for susceptibility against 140 isolates of *K. pneumoniae* (n=66), *S. aureus* (n=35), *E. coli* (n=15), and *P. aeruginosa* (n=6) and the percent of resistance were 100% in *K. pneumoniae* and *P. aeruginosa* while 96% in *E. coli* against AM. However, the resistance percent against these bacteria were G (76.3, 83.4 and 69.2%), PIP (2.1, 1.42 and 2.3%), CP (3.45, 3.12 and 3.76%), CTX (40.4, 45 and 48.2%), CIP (68.4, 76.4 and 74.7%) and CEF (28.7, 32.5 and 20.3%) for *K. pneumoniae*, *E. coli* and *P. aeruginosa* respectively.

Table (4) Antimicrobial Sensitivity Pattern (Resistant No.) and Percent (%) of 1178 Bacterial Genera Isolated from Different Clinical Sources

Antibiotics	Number (No.) and Percent Rate (%) of Resistant Isolates									
	E. coli n=417 (%)		*S. aureus* n=377 (%)		*K. pneumoniae* n=212 (%)		*P. aeruginosa* n=145 (%)		*Streptococcus* spp n=27 (%)	
	R**	S**	R	S	R	S	R	S	R	S
AMC*	22 (5.27)	395 (94.73)	120 (31.83)	257 (68.17)	20 (9.43)	192 (90.57)	7 (4.82)	138 (95.18)	16.2 (60)	10.8 (40)
AK	307 (73.62)	110 (26.38)	302 (80.1)	75 (19.9)	178 (83.96)	34 (16.04	119 (82.06)	26 (17.94)	27 (100)	0 (0)
AM	31 (7.43)	386 (92.57)	132 (35.01)	245 (64.99)	159 (75)	53 (25)	NA***	NA	0 (0)	27 (100)
AZM	417 (100)	0 (0)	377 (100)	0 (0)	NA	NA	NA	NA	NA	NA)
ATM	199 (47.72)	218 (52.28)	21 (5.57)	356 (94.43)	23 (10.84)	189 (89.16)	95 (65.51)	50 (34.49)	0 (0)	27 (100)
CAR	195 (46.77)	222 (53.23)	140 (37.13)	237 (62.87)	96 (45.28)	116 (54.72)	103 (71.03)	42 (28.97)	NA	NA
CF	224 (53.71)	193 (46.29)	194 (51.45)	183 (48.55)	71 (33.49)	141 (66.51)	64 (44.13)	81 (55.87)	NA	NA
CEC	0 (0)	417 (100)	168 (44.56)	209 (55.44)	0 (0)	212 (100)	37 (25.51)	108 (74.49)	27 (100)	0 (0)
CFZ	46 (11.03)	371 (88.97)	67 (17.77)	310 (82.23)	NA	NA	NA	NA	NA	NA
CD	417 (100)	0 (0)	139 (36.87)	238 (63.13)	NA	NA	NA	NA	27 (100)	0 (0)
CFM	89 (21.34)	328 (78.66)	47 (12.46)	330 (87.54)	0 (0)	212 (100)	18 (12.41)	127 (87.59)	NA	NA
CPO	130 (31.25)	287 (68.75)	308 (81.69)	69 (18.31)	0 (0)	212 (100)	NA	NA	NA	NA
CP	334 (80.09)	83 (19.91)	54 (14.32)	323 (85.68)	0 (0)	212 (100)	74 (51.03)	71 (48.97)	NA	NA
FOX	139 (33.33)	278 (66.67)	215 (57.02)	162 (42.98)	0 (0)	212 (100)	74 (51.03)	71 (48.97	0 (0)	27 (100)
CPR	417 (100)	0 (0)	162 (42.97)	215 (57.03)	148 (69.81)	64 (30.19)	74 (51.03)	71 (48.97)	9 (33.33)	18 (66.67)
CXM	60 (14.38)	357 (85.62)	110 (29.17)	267 (70.83)	141 (66.5)	71 (33.5)	0 (0)	145 (100)	NA	NA
KF	0 (0)	417 (100)	113 (29.97)	264 (70.03)	106 (50)	106 (50)	0 (0)	145 (100)	NA	NA
C	289 (69.3)	128 (30.7)	203 (53.84)	174 (46.16)	47 (22.16)	165 (77.84)	53 (36.55)	92 (63.45)	NA	NA
CN	125 (29.97	292 (70.03)	0 (0)	377 (100)	212 (100)	0 (0)	0 (0	145 (100)	NA	NA
CIP	192 (46.04)	225 (54.05)	157 (41.64)	220 (58.36)	120 (56.6)	92 (43.4)	42 (28.96)	103 (71.04)	18 (66.66)	10.8 (33.34)
CLM	83 (19.9)	334 (80.1)	189 (50.13)	188 (49.87	71 (33.49)	141 (66.51)	0 (0)	145 (100)	NA	NA
DA	NA	417 (100)	287 (76.12)	90 (23.88)	NA	NA	NA	NA	0 (0)	27 (100)
CT	NA	NA	NA	NA	NA	NA	0 (0)	145 (100)	NA	NA
E	417 (100)	0 (0)	170 (45.09)	207 (54.91)	NA	NA	0 (0)	145 (100)	13 (48.14)	14 (51.86)
G	182 (43.64)	235 (56.36)	242 (64.19)	135 (35.81)	148 (69.81)	64 (30.19)	16 (11.03)	129 (88.97)	NA	NA
GIP	NA	NA	283 (75.06)	94 (24.94)	NA	NA	145 (100)	0 (0)	NA	NA
IPM	382 (91.6)	35 (8.4)	308 (81.69)	69 (18.31)	199 (93.86)	13 (6.14)	118 (81.37)	27 (18.63)	13 (48.14)	14 (51.86)
DP	NA	NA	0 (0)	377 (100)	0 (0)	212 (100)	NA	NA	NA	NA
NAF	NA	NA	226 (59.94)	151 (40.06)	NA	NA	NA	NA	NA	NA
NA	245 (58.75)	172 (41.25)	377 (100)	0 (0)	85 (40.09)	127 (59.91)	42 (28.96)	103 (71.04)	0 (0)	27 (100)
F	397 (95.2)	20 (4.8)	168 (44.56)	209 (55.44)	56 (26.41)	156 (73.59)	110 (75.86)	35 (24.14)	NA	NA
NOR	0 (0)	417 (100)	377 (100)	0 (0)	13 (6.13)	199 (93.87)	95 (65.51)	50 (34.49)	NA	NA
OX	209 (50.11)	208 (49.89)	0 (0)	377 (100)	212 (100)	0 (0)	NA	NA	20 (74.07)	7 (25.93)
PG	NA	NA	0 (0)	377 (100)	148 (69.81)	64 (30.19)	114 (78.62)	31 (21.38)	NA	NA
PIP	NA	NA	377 (100)	0 (0)	164 (77.35)	48 (22.65)	37 (25.51)	108 (74.49)	14 (51.85)	13 (48.15)
RA	192 (46.04)	225 (53.96)	316 (83.81)	61 (16.19)	42 (19.81)	170 (80.19)	67 (46.2)	78 (53.8)	NA	NA
TE	79 (18.94)	338 (81.06)	118 (31.29)	259 (68.71)	186 (87.73)	26 (12.27)	59 (40.68)	86 (59.32)	27 (100)	0 (0)
TC	0 (0)	417 (100)	63 (16.71)	314 (83.29)	82 (38.67)	130 (61.33)	NA	NA	0 (0)	27 (100)

TOB	156 (37.41)	261 (62.59)	377 (100)	0 (0)	8339.15)	129 (60.85)	13 (8.96)	132 (91.04)	NA	NA
SXT	56 (13.42)	361 (86.58)	203 (53.84)	174 (46.16)	201 (94.81)	11 (5.19)	134 (92.41)	11 (7.59)	9 (33.33)	18 (66.67)
VA	417 (100)	0 (0)	362 (96.02)	15 (3.98)	170 (80.18)	42 (19.82)	60 (41.37)	85 (58.63)	NA	NA

Key *: Abbreviation were taken from Table (2); **: R: Resistant; S: Sensitive; ***NA: Not Applicable.

While for *S. aureus*, the resistance rates were AM 85.56%, E 49.12%, KF 34.3%, G 45%, VA 0.00%, CIP 42.41%, and AK 15.23%. Egbebia and Famurewa [19] they studies on 970 samples which collected from urine, high vaginal swab, blood, ear, sputum, pus, cerebrospinal fluid, semen, stool and nasal fluids. Among of all samples they detected 544 isolates of *K. pneumoniae* (56.1%), when 120 isolates (96%) resist to CFM, 117 (93.6%) to ATM, 109 (87.2%) to CTX, and 106 (84.4%) to CXM. Also Younis [20] reported that 397 samples (13.8%) are positive growths of bacterial genera among 2872 patients were admitted with clinical diagnosis of neonatal sepsis. *E. coli* comprise with 48 (12.1%), *K. pneumoniae* 40 (10%), *S. aureus* 29 (7.3%), *P. aeruginosa* 14 (3.5%) and *Streptococcus* spp 9 (2.3%) among all of 2872 samples. He reported that the resistance percent of AMP were 73, 93, 90, 86 and 56%, G 64.5, 62, 60, 71 and 66%, AK 19, 21, 22.5, 29 and 22%, CEF 37.5, 31, 45, 43 and 33%, CFT 42, 41, 55, 86 and 22%, CTX 29, 31, 32.5, 43 and 22%, IP 0, 10, 2.5, 28.5 and 11% for *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *Streptococcus* spp and the rates of resistances of VA 3.4 and 0% for *S. aureus* and *Streptococcus* spp, while the rates of CIP were 42, 32 and 36% for *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

The cause of the multi–drug resistant among bacterial strains due to: First, including the community acquired bacterial isolates along with hospital isolates would have provided a much better picture of resistance patterns of strains in this geographical area. Second, molecular typing and plasmid profile of the bacterial isolates would provide the much needed details about the strains and lastly extended spectrum beta lactam (ESBL) which have become a major cause of nosocomial infections with MDR strains should be analyze [21].The rapid spread of bacteria expressing multidrug resistance (MDR) has necessitated the discovery of new antibacterial and resistance modifying agents [22]. Since the initial discovery of bacterial efflux pumps in the 1980s, many have been characterized in community and hospital acquired pathogens [23]. Efflux pumps are able to extrude structurally diverse compounds, including antibiotics used in a clinical setting, rendering the drugs therapeutically ineffective [24].

Antibiotic resistance can develop rapidly through changes in the expression of efflux pumps. It is, therefore, imperative that new antibiotics, resistance modifying agents and, more specifically, efflux pump inhibitors (EPIs) are characterized [25]. The use of bacterial resistance modifiers such as EPIs could facilitate the re-introduction of therapeutically ineffective antibiotics back into clinical use and might even suppress the emergence of MDR strains [23].In the other hand, Ghafourian *et al.*, [26] isolated and identified 113 isolates of *K. pneumoniae* which taken from respiratory tract infections (RTIs), 67 isolates of them produce extended spectrum beta lactamase (ESBL) and 46 isolates not produce extended spectrum beta lactamase (non–ESBL). They found that 19 isolates (28.3%) resist to AK, 67 (100%) to ATM, 62 (92.5%) to CFT, 46 (68.6%) to CTX, 11 (16.4%) to CIP, 62 (92.5%) to CEF and 0.00% to IP. Chinwe and Ezeronye [8] worked on susceptibility tests on 80 isolates of *S. aureus* and used nine antibiotics for this purpose, and the their results shown 80 isolates (100%) resist to PG, 77 (96.3%) to AM, 27 (33.8%) to OX, 24 (30%) to CFT, 23 (28.8%) to CFM, 14 (17.5%) to E, 31 (38.5%) to G, 56 (70%) to TE and 30 (37.5%) to C. Schito *et al,*. [27] reported in their research that among 2315 isolates of *E. coli*, 48.3% show resistance to AMP, 3.8% to AM, 2.4% to CXM, 8.6% to NA, 8.1% to CIP, 29.4% to SXT and 1.6% to F.

The bacterial isolates (n=1178) were classified and grouped in to 23 groups according to their resistances through antimicrobials which used in this study, as clarified in Table (5) and known as Antibiogram groups. This Antibiogram table of antimicrobial resistance for bacterial isolates demonstrated that the predominant mode which include more resistant isolate represented in mode (1), which was resist to 85.36% of all antimicrobials, except FOX, CPR, PIP, RA, TE and VA, while the resistance Antibiogram pattern of remained groups ranged between 43.9% – 78.04%.

Table (5) Antibiogram Groups and Resistance Percent to Antimicrobial Agents

Antibiogram Groups		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
No. of Isolates		11	27	102	221	78	63	11	29	45	91	187	23	1	39	79	32	15	26	2	62	18	6	10
No. of B. Each Genera	*E. coli*	4	11	38	92	19	28	2	9	6	24	90	3	0	15	27	9	3	8	0	20	6	2	1
	S. aureus	2	3	41	46	28	13	5	15	21	36	56	12	0	9	24	13	8	11	1	21	5	2	5
	K. pneumoniae	3	8	22	28	23	9	3	2	6	19	25	5	0	6	16	8	3	5	0	12	6	1	2
	P. aeruginosa	1	3	1	49	7	11	1	2	12	8	14	3	1	8	9	2	1	2	1	5	1	1	2
	Streptococcus spp	1	2	0	6	1	2	0	1	0	4	2	0	0	1	3	0	0	0	0	4	0	0	0
% of Resistance		85.36	78.04	73.17	65.85	63.41	63.41	63.41	60.97	60.97	56.09	56.09	56.09	56.09	56.09	53.65	53.65	53.65	53.65	53.65	51.21	43.9	43.9	43.9
Antibiotics	AMC*	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+
	AK	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AM	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
	AZM	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-
	ATM	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	-	+
	CAR	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-
	CF	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CEC	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+
	CFZ	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
	CD	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+
	CFM	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	CPO	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
	CP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-
	FOX	-	-	+	+	-	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
	CPR	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
	CXM	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-
	KF	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CN	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
	CIP	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-
	CLM	+	+	+	-	-	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-
	DA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	CT	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
	E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	G	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-
	GIP	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	IPM	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+
	DP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	NAF	+	+	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
	NA	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
	F	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	NOR	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
	OX	+	+	-	-	+	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-
	PG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	PIP	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	RA	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-
	TE	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TC	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
	TOB	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	SXT	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	VA	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	+	+

Key * = Abbreviation were taken from Table (2); + = Resistant; - = Sensitive.

References

1. Okonko N.A., Lennox J.A., Adewale O.G., Motayo B.O., et al., (2010). "Survey of the Efficacy and Quality of Some Brands of the Antibiotics Sold in Calabar Metropolis, South Region of Nigeria". Elect. J. Environ., Agricult. Food Chem., 9 (7): 1232–1248.
2. Heffernan H., Wong T.L., Lindsay J., Bowen, B. and Woodhouse, R., (2011). "A Baseline Survey of Antimicrobial Resistance in Bacteria from Selected New Zealand Foods 2009–2010, Antibiotic Reference Laboratory". Institute of Environmental Science and Research, MAF's (Ministry of Agriculture and Forestry) Agricultural Compounds and Veterinary Medicines (ACVM), pp. 4–5.
3. Uwaezouke J.C. and Aririatu, L.E., (2004). "A Survey of Antibiotic Resistant *Staphylococcus aureus* Strains from Clinical Sources in Owerri".J. Appl. Sci. Environ., 8 (1): 67–69.
4. Fluit A.C., Jones M.E, Schmitz F.J., Acar J., et al., (2000). "Antimicrobial Susceptibility and Frequently of Occurrence of Clinical Blood Isolates in Europe from the SENTRY Antimicrobial Surveillance Program, 1997 and 1998". Clin. Infect. Dis., 30: 454–460.
5. Sahm D.F., Thornsberry C., Mayfield D.C., Jones M.E. and Karlowsky J.A., (2001). "Multi–Drug Urinary Tract Isolates of *Escherichia coli*: Prevalence Demographics in the United States in 2000". Antimic. Ag. Chemo., 45: 1402–1406.
6. Stelling J.M., Travers K., Jones R.N., Turner P.J., et al., (2005). "Integrating *Escherichia coli* Antimicrobial Susceptibility Data from Multiple Surveillance Programs". Emerge. Infect. Dis., 11 (6): 873–882.
7. Blondeau J.M., (2004). "Current Issues in the Management of Urinary Tract Infections: Extended Release Ciprofloxacin as Novel Treatment Option". Drugs, 64: 611–628.

8. Chinwe C.O. and Ezeronye O.U., (2003). "Antibiotic Resistant *Staphylococcus aureus* in Abia State of Nigeria". Afr. J. Biotech., 2 (10): 374–378.
9. Akindele A.A., Adewuyi I.K., Adefioye O.A., Adedokun S.A. and Olaolu A.O., (2010). "Antibiogram and Beta Lactamase Production of *Staphylococcus aureus* Isolates from Different Human Clinical Specimens in a Tertiary Health Institution in Ile-Life, Nigeria". Amer. – Euras. J. Sci. Res., 5 (4): 230–233.
10. Kayser F.H., Bienz K.A., Eckert J. and Zinkernagel R.M., (2005). "Medical Microbiology". 1st ed. London: Blackwell Science Ltd., pp.278–282.
11. Toroglu S. and Keskin D., (2011). "Antimicrobial Resistance and Sensitivity among Isolates of *Klebsiella pneumoniae* from Hospital Patients in Turkey". Intern. J. Agricult. Bio., 13 (6): 941–946.
12. Arora D., Jindal N. and Romit R.K., (2011). "Antibiotic Resistance in *Pseudomonas aeruginosa* Challenge". *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(2): 82–84.
13. Vives F.M. and Garnica D., (2006). "Comparison of Virulence between Clinical and Environmental *Pseudomonas aeruginosa* isolates". Internat. Microb., 9: 247–252.
14. Loureiro M.M., de Moraes B.A., Mendonça V.L., Quadra M.R., et al., (2002). "*Pseudomonas aeruginosa*: Study of Antibiotic Resistance and Molecular Typing in Hospital Infection Cases in a Neonatal Intensive Care Unit from Rio de Janeiro City, Brazil". Memórias do InstitutoOswaldo Cruz, 97(3): 387–394.
15. Cavalieri S.J., Rankin I.D., Harbeck R.J., Sautter R.L., et al., (2005). "Manual of Antimicrobial Susceptibility Testing. Library of Congress Cataloging in Publication Data". American Society for Microbiology, pp. 168.
16. Hardie J.M. and Whiley R.A., (1997). "Classification and Overview of the Genera *Streptococcus* and *Enterococcus*". J. Appl. Microb. Sym. Supp., 83: 1–11.
17. National Committee for Clinical Laboratory Standards (NCCLS), (2013). "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard". 4th ed. Document VET01 – A4. USA, 33(7): 1–17.
18. Desai K.J. and Malek S.S., (2009). "Neonatal Septicemia: Bacterial Isolates and Their Antibiotics Susceptibility Patterns". Nat. J. Int. Res. Med., 1 (3): 12–15.
19. Egbebia O. and Famurewa O., (2011). "Antibiotic Resistance of *Klebsiella* Isolated from Some Hospitals in South West, Nigeria to Third Generation Cephalosporins". Adv. Trop. Med. Pub. Heal. Intern., 1 (3): 95–100.
20. Younis N.S., (2011). "Neonatal Sepsis in Jordan: Bacterial Isolates and Antibiotic Susceptibility Patterns". Rawal Med. J., 36 (3): 1–16.
21. Anil, C., and Shahid, R.M., (2013). Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* Clinical isolates at a Tertiary Care Hospital in Kathmandu, Nepal. Asian J. Pharma. Clin. Res., 6(3): 235–238.
22. Tariro, A.C., and Stanley, M., (2011). *In Vitro* Antibacterial Activity of Selected Medicinal Plants from Zimbabwe. The African J. Plant Sci. Biotech., 5 (1): 1–7.
23. Stavri, M., Piddock, L.V.J., and Gibbons, S., (2007). Bacterial efflux pumps inhibitors from natural sources. J. Antimicrob. Chemotherap., 59: 1247–1260.
24. Amusan, O.O.G., Sukati, N.A., Dlamini, P.S., and Sibandze, F.G., (2007). Some Swazi phytomedicines and their constituents. African J. Biotech., 6:267–272.
25. Stermitz, F.R., Lorenz, P., and Tawara, J.N., (2000). Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. Proceedings of the National Academy of Sciences USA, 97: 1433–1437.
26. Ghafourian S., Bin – Shekawi Z., Sadeghifard N., Mohebi R., et al., (2011). "The Prevalence of ESBLs Producing *Klebsiella pneumoniae* Isolates in Some Major Hospitals, Iran". The Open Microb. J., 5: 91–95.
27. Schito G.C., Naber K.G., Botto H., Polou J., et al., (2009). "The ARESC (Antimicrobial Resistance Epidemiological Survey on Cystitis) Study: An International Survey on the Antimicrobial Resistance of Pathogens involved in Uncomplicated Urinary Tract Infections". Intern. J. Antimic. Ag., 34 (5): 4–12.

مضادات النمط الحيوي لبكتيريا مختلفة معزولة من مصادر سريرية مختلفة

ريبوار محمد حمه صالح ، خديجه خليل مصطفى ، زيرك فقى احمد عبدالرحمن

قسم علوم الحياة ، كلية التربية ، جامعة صلاح الدين ، اربيل ، العراق

الملخص

تم اخذ و تشخيص 1178 عزلة من انواع مختلفة لبكتيريا من اشخاص مصابين من مصادر مختلفة تضمنت البول (عدد 641)، مسحة (عدد 483)، جروح (عدد 29) والدم (عدد 25)، لقد أكدت الدراسة الحالية على مقاومة المضادات الحيوية ضد بعض الأجناس البكتيريا المرضية و التي شملت*E. coli* (عدد 417) و*S. aureus* (عدد 377) و *K. pneumonia* (عدد 212) و *P. aeruginosa* (عدد 145) و *Streptococcus* spp. (عدد 27) والتي أجريت في المختبر الداخلي من المستشفى التعليمي في مدينة أربيل، في 15 من سبتمبر 2012 الى 20 يونيو 2013, ولقد تم تحديد الحساسية عن طريقة الانتشار القرصي الموصى به من قبل (NCCLS). اضافة الى ذلك تم اختبار المضادات الحيوية التالية: اموكسيكلاف AMC (20µg) و اميكاسين AK (30µg) و اموكسيلين AM (30µg) و ازتروميسين AZM (15µg) و ازترونام ATM (30µg) و كاربينسيلين CAR (100µg) و سيفاكلور CF (30µg) و سيفوتاكسيم CEC (30µg) و سيفازولين FZ (15µg) و سيفدينير CD (5µg) و سيفيكزيم CFM (30µg) و سيفونيسيد CPO (30µg) و سيفوبيرازون CP (75µg) و سيفوكزيتين FOX (30µg) و سيفبروزيل CPR (30µg) و سيفوراكسيم CXM (30µg) و سيفالوثين KF (30µg) و كلورامفيكول C (30µg) و سينوكزاسين و سيبروفلوكساسين CIP (5µg) و كلاريثرومايسين CLM (15µg) و كليندامايسين DA (2µg) و كوليستين CT (10µg) و ارثرومايسين E (15µg) و جنتامايسين G (10µg) و جريبافلوكساين GIP (5µg) و اميبينيم IPM (10µg) و مثيسيلين DP (5µg) و نافسيلين NAF (1µg) وحامض ناليديكسيك NF (30µg) و نايتروفيورانتن F (300µg) و اوكساسيلين OX (1µg) و بنسيلين ج PG (10µg) و بايبرسيلين PIP (100µg) و ريفامبين RA (5µg) و تتراسايكلين TE (30µg) و تيكارسيلين TC (75µg) و توبرامايسين TOB (10µg) و ترايمثوبريم–سلفاميثوكسازول SXT (1.25 / 23.75µg) و و فانكومايسين VA (30µg).لقد اظهرت نسبة المقاومة لجميع العزلات البكتيرية مديات مختلفة من المقاومة والتي بدأت من 0.00٪ إلى 100٪.

صنفت العزلات البكتيرية (عدد 1178) الى 23 مجموعة وفقا لمستويات المقاومة للمضادات الحيوية و التي استخدمت في هذه الدراسة، كما هو موضح في الجدول (5) والمعروفة باسم مضادات النمط الحيوي. يوضح الجدول مقاومة انواع البكتريا لهذه المضادات الحيوية, الطريقة السائدة و التي تشمل العزلات الاكثر مقاومة ممثلة في الوضع (1)، و المقاومة لـ 85.36٪ من جميع مضادات الحيوية، عدا FOX و CPR و PIP و RA و TE و VA، بينما تراوح نمط مقاومة المضادات للمجموعات المتبقية بين 43.9٪ 78.04٪.