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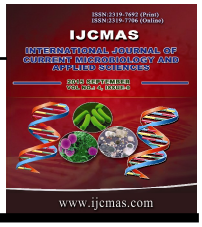

Original Research Article

What remains against carbapenem-resistant Enterobacteriaceae and Acinetobacter spp? Evaluation of Tigecycline and Colistin

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Keywords

Carbapenam resistant *Enterobacteriacae*, *Acinetobacter spp*, Colistin, Tigecycline

A B S T R A C T

Nosocomial infections with multi-drug resistant bacteria (MDR) e.g: Carbapenem-resistant *Enterobacteriaceae* (CRE) *and Acinetobacter* species are increasing in our hospitals with limited therapeutic options , so increased mortality and morbidity. The aim of this study to determine the *in vitro* activity of Tigecycline (TIG) and Colistin (COL) against clinical isolates of CRE and *Acinetobacter spp* in Tanta University Hospitals, Egypt. A total of 47 CRE and 30 *Acinetobacter spp* were isolated between September 2014 to April 2015. Susceptibility done by disk diffusion method (DD) for all antibiotics and minimal inhibitory concentration (MIC) for TIG and COL by Etest.Using E test, COL was sensitive against (100%, MIC(50/90) : 0.5 /1.5 μg/mL) of *Acinetobacter spp* , (80.9%, MIC(50/90): 0.5 /2 μg/mL) of *Enterobacteriacae* . Sensitivity of TIG was (97.9%, MIC(50/90): 0.5/1 μg/mL) for *Enterobacteriacae* and (60%, MIC(50/90): 2/4 μg/mL) for *Acinetobacter spp.* Although COL appears to be a good choice for treatment of CRE and *Acinetobacter spp,* its toxicity limit its use. TIG is safer and promising in *Enterobacteriacae* treatment but there is resistance in *Acinetobacter spp*.

Introduction

Nosocomial infection by multi-drug resistant bacteria (MDR) e.g: extended-spectrum-Beta-lactamase(ESBL) producing *Enterobacteriaceae*, *Acinetobacter baumannii* and carbapenem-resistant *Enterobacteriaceae* (CRE) is increased worldwide, with major burden due to limited choices of antibiotics (Peleg & Hooper, 2010, Magiorakos et al.,2012). Tigecycline (TIG) and Colistin (COL) are examples of the few drugs that are available for treatment of these resistant pathogens (Giamarellou &Poulakou, 2009).

COL which is synthesized by Bacillus polymyxa, act by interfering with the function of cytoplasmic membrane of bacteria. So it is used as a bactericidal drug against Gram-negative bacteria (Hancock & Chapple, 1999, Akalin, 2007). It was firstly introduced in 1952, however its systemic use is stopped due to its toxicity especially nephrotoxicity and neurotoxicity (Catchpole et al.,1997, Yow et al., 1961). The

emergence of MDR gram-negative bacteria especially Acinetobacter *spp* with lack of new antibiotics lead to the reuse of polymexins especially COL (Gales et al., 2001).

TIG is the first member of Glycylcyclines group, it act on ribosomes by inhibition of protein synthesis (European Medicines Agency Tygacil, tigecycline). TIG has fewer side effects in comparison with other antimicrobials available for treatment of MDR bacteria e.g: polymexins and aminoglycosides (Rello, 2005, Ku et al.,2012). However TIG still has its limitation as it reaches low serum level and recent studies from the U.S. Food and Drug Administration (FDA) evidenced increased mortality risk in patients receiving TIG (FDA, 2010, Tasina et al., 2011, Yahav et a.l, 2011). Due to the lack of treatment options it is still used with the same clinical efficacy of the other available drugs (Ku et al., 2012).

MDR bacteria mainly CRE and *Acinetobacter spp* are widely spread in our hospitals. So the aim of this study to evaluate and compare the in vitro activity of COL and TIG in treatment of these MDR bacteria that are collected from patients admitted to ICUs of our University hospitals.

Materials and Methods

Collection of bacteria:

This study was conducted from September 2014 to April 2015 at Tanta University hospitals, Egypt with prior approval from Institutional Ethical Committee. Forty seven isolates of *Enterobacteriaceae* were studied: *Klebsiella pneumoniae* (11 strains), *Klebsiella spp.* (11 strains), *Enterobacter spp* (20strains), *Citrobacter spp* ((4 strains), *Serratia marcescenes* (one strain) and *Acinetobacter spp* (30 isolates).Bacterial isolates were identified by conventional methods according to standard laboratory methods.These bacterial isolates were randomly collected from different departments of our hospitals (emergency, neonatal, pediatric Intensive Care Units, general surgery and urology wards).

Susceptibility to antibiotics:

All susceptibility testing and interpretations were performed according to Clinical and Laboratory Standards Institute guidelines 2014 (CLSI, 2014), by Kirby-Bauer's disc diffusion (Oxoid, Thermofisher Scientific, UK) on Mueller-Hinton agar plates. The discs used were Ceftazidime, Cefepime, Cefoperazone, Amikacin, Gentamicin, Tobramycin, Ciprofloxacine, Imipenem, Meropenem, Amoxicillin/Clavulinic and Ampicillin. Strains that were resistant to Imipenem or Meropenem or both were selected for Modified Hodge test (MHT) as recommended by Clinical and Laboratory Standards Institute (CLSI, 2014 guidelines).

Susceptibility to TIG and COL

The susceptibility tests for TIG and COL were performed for CRE and *Acinetobacter spp* by Kirby-Bauer's disc diffusion (Oxoid discs, UK) containing 15 and 10 µg respectively and E tests (BioMérieux, France). *E. coli* ATCC 25922 was used as quality control strain.

The disc zone diameters of COL were interpreted according to National Committee for Clinical Laboratory Standards(NCCLS) 1981 guidelines: resistant $\leq$ 8 mm; susceptible $\geq$ 11 mm and the product literature (criteria of Gales et al., 2011): resistant $\leq$ 11 mm; susceptible $\geq$14 mm with MIC breakpoints of CLSI 2014: resistant $\geq$4 µg/ml ; sensitive $\leq$ 2 µg/ml.

Since no interpretive criteria have been approved for TIG in CLSI, the results were interpreted by U.S. FDA for *Enterobacteriaceae* (Tygacil package insert [June, 2005], Wyeth Pharmaceuticals, Inc., Philadelphia, PA), against *Acinetobacter spp* the results were interpreted by the criteria recommended by Jones *et al.* (2007) and measures used by U.S.FDA for *Enterobacteriacae,* table 1. (Liu et al.,2012)

Statistical analysis

Data were analyzed using statistical package of social science (SPSS) version 21.Qualitative data were described using number and percent. MIC required to inhibit 50% and 90% of the strains (MIC50 and MIC90, respectively) were calculated using Microsoft- Excel- 2007 software

Results and Discussion

During 9 month period a total of (47) CRE and (30) CR *Acinetobacter spp* were isolated. 53% of isolated CRE and *Acinetobacter spp* were from neonatal and pediatric ICU.CRE isolated predominately in the age group less than one year (53%) and (40%) of *Acinetobacter spp* isolated from the age group 19-65y. 55.3% of CRE isolates from blood specimens, 73% of *Acinetobacter spp* from endotracheal aspirates. 85.1% and 93% of CRE and *Acinetobacter spp* were positive for MHT(Table 2).

By using DD, the antimicrobial susceptibility profile was as follow: all isolates were resistant to Ceftazidime, Cefoperazone, Cefepime, Amoxicillin/Clavulinic and Ampicillin. Of tested aminoglycosides Gentamycin show the better sensitivity (44.7%) for CRE and Tobramycin (23.3%) for *Acinetobacter spp.* Senstivity of TIG was 43.3% (13) for *Acinetobacter spp* and 97.9% (46) in CRE (only one strain of *K.spp* has intermediate sensitivity by DD method and was resistant by E test but this strain was sensitive to COL). For COL sensitivity by using NCCLS1981 measures the sensitivity was (100%, 83 %) for *Acinetobacter spp* and CRE, however by application of Gales criteria it is decreased to (70%, 19.1%) for both isolates respectively (Table 3).

Sensitivity of COL was (80.9%, 100%) in *Enterobacteriacae* and *Acinetobacter spp* respectively. Nine strains (19.1%) of *Enterobacteriacae* were resistant to COL but sensitive to TIG (3 isolates of *K.pneumoniae,* 3 *Enterobacter spp,* 2 *Citrobacter spp,* one strain of *Serratia marscenes).* TIG sensitivity was 97.9% (46) in *Enterobacteriacae* but was 60% (18) for *Acinetobacter spp* (Table 4).

The high prevalence of MDR *Enterobacteriacae* and *A.baumannii* is a big clinical problem due to their resistance to all most antimicrobials e.g: β-lactams, carbapenams, aminoglycosides and quinolones with limited treatment options to COL or TIG (Kanj & Kanafani, 2011, Peerayeh et al.,2014). There is limited resources about the situation of CRE and MDR *A.baumanii* in Egypt so there is limited data about the selection of appropriate antimicrobials for treatment. So this study was conducted to evaluate the in vitro sensitivity of COL and TIG against CRE and *Acinetobacter spp* in Tanta University Hospitals, Egypt.

The results have shown that CRE were more frequently isolated from blood stream infections 55.3 % (26/47) especially from neonatal and pediatric ICU 80.8% (21/26), followed by wound swabs (25.5%) and end tracheal aspirates (19%). This was supported by another study in Alexandria that found 54

% of CRE isolated from blood stream infections especially from neonatal ICU (Shawky et al., 2015). Also Souli et al, 2010 isolate most of *Klebsiella pneumoniae* carbapenemases (KPCs) from bacteraemic cases and a systemic review of KPCs reported that the most common site of this infection was blood (52%) (Lee et al., 2012). For *Acinetobacter spp*, 73.3% (22/30) isolated from end tracheal aspirates, 20% (6/30) from blood, 6.7% (6/30) from urine. This was consistent with another study, in which the first site for isolation of *Acinetobacter spp* was the lower respiratory tract (11 cases), blood (7 cases) (Tankovic et al.,1994) also a review article for *Acinetobacter* infection in the Intensive Care Unit, concluded that *Acinetobacter* can infect any body site, mainly the lower respiratory tract (42%), the bloodstream (18%), and the urinary tract (10%) (Rungruanghiranya et al.,2005).

As regard the type of isolated *Enterobacteriaceae*, 42.65 % (20/47) of CRE isolates *were Enterobacter spp*., 23.4 % (11/47) were *k.pneumonae*, 23.4% (11/47) were *K.spp*. Also Kiedrowski *et al*.(2014) found that *Enterobacter cloacae* was the most common CRE isolated. However Schechner et al found that *k.pneumonae* was the most common CRE isolated and Jacob et al isolate most of CRE from *k.spp* (49), followed by *Enterobacter* species (14) and *E. coli* (nine) (Schechner et al.,2009, Jacob et al., 2013).

In the present study most of isolates are resistant to different antimicrobial classes. However 51% (24/47) of CRE are sensitive to aminoglycosides.The best in vitro activity in CRE was for Gentamycin 44.7% (21/47), followed by Amikacin 6.4 % (3/47), but there was no sensitive strains to Tobramycin. Gentamycin has better activity against isolates obtained from neonatal blood stream infections 52.4% (11/21) especially *K.spp* (8 isolates) and *K.pneumoaniae* (7 isolates). This finding was in agreement Shawky *et al.* (2015) that found the better activity for the tested aminoglycosides was for Gentamycin (28%), followed by Amikacin (22.3%). Also Castanheira *et al.*(2008) support the same finding with better sensitivity for Gentamycin (58.3%) followed by Amikacin (53.3%) for CRE.

Regarding *Acinetobacter spp* there was better sensitivity to Tobramycin 23.3% (7/30), followed by Gentamycin 16.7% (5/30), with no sensitive strains to Amikacin. This was in agreement with Ashour *et al.* (2009) and Gündeşlioğlu *et al*. (2014) in which the isolated *Acinetobacter* show better sensitivity to Tobramycin (54.4%, 53.8%), followed by Gentamycin (42.6%, 31.8%), however sensitivity for Amikacin in these two studies was (44.9%, 34.7%) respectively as the antibiotic sensitivity done by Vitek-2 Compact automated system. In contrast Ahmed *et al*.(2012) and Al Mobarak *et al*. (2014) reported better sensitivity of *A.baumanii* to amikacin (78%, 15.4%) than gentamycin (9.4%, 9.7 %) respectively. Anyway resistance of pathogens to common antibiotics have increased nowadays and the antibiotic susceptibilities differ from one country to another even from one institute to another according to the applied antibiotic policies.

Reuse of COL appear nowadays due to wide spread of MDR organisms in spite of its toxicity with absence of new effective and safer drugs. Additionally no interpretative breakpoints has been identified by CLSI but European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015) set the breakpoints for enterobacteriacae and resistance for colistin at $\geq$ 4 g/mL. In the

current study 80.9% (38/47) of *Enterobacteriacae* and 100% of *Acinetobacter spp* was sensitive to COL by E test. The continued use of polymexins, especially as a selective digestive decontaminant resulted in the development of secondary resistance to COL especially among *Enterobacteriacae* (Lübbert et al.,2015). The resistance rates to COL varied in this study from 100% (1/1) for *Serratia marcescenes*, 50% (2/4) for *citrobacter spp*, 27.3 % (3/11) for *K.pneumoniae*, 15% (3/20) for *Enterobacter spp* with no resistance in *K.spp* and *Acinetobacter spp* isolates.

Lo-Ten-Foe *et al*. (2007) reported resistance by broth microdilution (BMD) test in *K.pneumoniae* 70% (7/10), *citrobacter freundii* 69% (9/13), 50% (1/2) *Acinetobacter spp, Enterobacter spp* 7% (1/13), no resistance in *K.spp*. Another study in Singapore found 31% of Gram-negative isolates were resistant to COL, *Serratia spp.* (9 ⁄ 9, 100%), *Enterobacter spp.* (15 ⁄ 20, 75%) and *Klebsiella spp.* (3 ⁄ 18, 17%) with no resistance was detected in *Acinetobacter spp.* and *E. coli.* (Tan & Ng, 2007).

Among *Enterobacteriacae* COL exhibited excellent activity against *K.spp* and *Enterobacter spp* (MIC_{90}= 2 μg/ml) and was less active against *K.pneumonia* (MIC_{90}= 16 μg/ml). Also Maalej et al reported good activity of COL against *E. cloacae* (MIC90 = 0.5 mg l^{-1}), in contrast to *K. pneumoniae* (MIC90 = 16 mg l^{-1}) (Maalej et al., 2011). However another study found better activity of COL against *K. pneumoniae* (MIC90 =≤ 1 μg/ml) in comparison with *Enterobacter spp* (MIC90 =16 μg/ml) (Tan & Ng, 2007). There are studies in different areas reported the resistance to this life saving antibiotic especially among *Enterobacteriacae* (Chen et al., 2011, Garbati et al., 2013). Although the sensitivity of *Acinetobacter spp* to colistin is 100% in most studies (Somily et al.,2012, Gündeşlioğlu et al., 2014, Peerayehet al., 2014). There are studies reported resistant strain for *A.baumanii* to COL that will return us to the pre antibiotic era and increase in mortality and morbidity (Mezzatesta et al., 2008, Arroyo et al.,2009, Chang et al., 2012).

In spite of COL effectiveness against MDR bacteria e.g: CRE, *Acinetobacter spp* and *Pseudomonas aeruginosa*, its use is limited due to its neurotoxicity and nephrotoxicity. This raises the use of TIG as a safer drug with low toxicity for treatment of MDR organisms. There are no breakpoints set by CLSI for TIG for *Enterobacteriaceae* but FDA approved sensitive breakpoint for *Enterobacteriaceae* is ≤ 2 g/mL).

In the current study sensitivity of *Enterobacteriaceae* to TIG was 97.9% (MIC(50/90), 0.5/1μg/mL) with one resistant strain only (*K.spp* isolate), but *Acinetobacter spp* sensitivity was lower 60% (18/30) (MIC(50/90), 2/4 μg/mL). Also a study conducted in a tertiary hospital in Alexandria, Egypt found no resistance among *E. coli* and *K. pneumoniae* isolates to TIG (MIC50 of 1 μg/ml) and was the most active antibiotic tested against these isolates but 40% of *A. baumannii* was resistant to TIG (MIC range was 0.5–16 mg/ml, MIC50 /MIC90 : 2 /16 μg/ml) (Mohamed & Youssef, 2011).The increased resistance of *Acinetobacter spp* to TIG can be explained by the over expression of the AdeABC multidrug efflux pump (Giamarellou et al., 2008).

The *In Vitro* Surveillance in Taiwan study when interpret TIG sensitivity by FDA criteria, was 96.32% for ESBL-producing *K. pneumoniae* (MIC90, 2 μg /ml) and decreased from 80.9% in 2006 to 55.3% in 2009 but increased to 73.4% in 2010 for *A. baumannii* (Chen et al.,2012).

Table.1 Interpretive MIC and disk diffusion interpretive criteria for Tigecycline applied in this study

MIC (µg/ml)				Diam (mm) by disk diffusion method		
Bacterium	S	I	R	S	I	R
Enterobacteriacae	≤2	4	≥8	≥19	15-18	≤14
Acinetobacter*	≤2	4	≥8	≥16	13-15	≤12

The interpretation of MICs in E tset and the diameters of the inhibitory zone in the disk diffusion testing were based on the criteria proposed by the U.S. Food and Drug Administration (FDA). * Jones criteria for Acinetobacter

Table.2 Patient and specimen data of isolated carbapenam resistant Enterobacteriacae and Acinetobacter spp

	Enterobacteriacae (47)	Acinetobacter spp (30)
Sex		
Male	25(53.2%)	16(53.3%)
Female	22(46.8%)	14(46.7%)
Age		
<1y	25(53.2%)	10(33.3%)
1-18years	-----	8(26.7%)
19-65years	22(46.8%)	12(40%)
Department		
NICU& PICU	25(53.2%)	16(53.3%)
EICU	11(23.4%)	14(46.7%)
General surgery	11(23.4%)	-------
Sample		
Blood	26(55.3%)	6(20%)
ETA	9(19.1%)	22(73%)
Wound swab	12(25.5%)	-------
Urine	------	2(6.7 %)
MHT		
Positive	40(85.1%)	28(93%)
Negative	7(14.9 %)	2(6.7%)
Species		
k.pneumonae	11(23.4%)	
K.spp	11(23.4 %)	
Enterobacter spp	20(42.6%)	--------
citrobacter spp	4(8.5 %)	
Serratia marcescens	1(2.1 %)	

NICU& PICU: Neonatal and Pediatric Intensive Care Units , EICU: Emergency ICU, ETA : endotracheal aspirate, MHT : modified hodge test

Table.3 Antimicrobial susceptibility pattern of carbapenem resistant Enterobacteriacae and Acinetobacter spp by disk diffusion method

	Enterobacteriacae (47)			Acinetobacter spp(30)		
	S	**I**	**R**	**S**	**I**	**R**
ColistinNCCLS1981*	39 (83 %)	8(17%)	0%	30(100 %)	0%	0%
ColistinGales**	9(19.1%)	21(42.%)	17(36.%)	21(70%)	6(20%)	3(10 %)
Tigecycline*	46(97.9%)	1(2.1 %)	0%	13(43.3%)	6(20%)	11(36.%)
Gentamycin	21(44.7%)	7(14.9%)	19(40.%)	5(16.7 %)	0%	25(83%)
Amikacin	3(6.4%)	8(17%)	36(76.%)	0%	3(10%)	27(90%)
Tobramycin	0%	1(2.1%)	46(97.%)	7(23.3%)	0%	23(76.%)
Ciprofloxacin	10(21.3%)	3(6.4 %)	34(72%)	3(10%)	3(10%)	24(80%)
Ceftazidime	0%	0%	47(100%)	0%	0%	30(100%)
Cefoperazone	0%	0%	47(100%)	0%	0%	30(100%)
Cefepime	0%	0%	47(100%)	0%	0%	30(100%)
Amoxicillin/Clavulinic	0%	0%	47(100%)	-	-	-
Ampicillin	0%	0%	47(100%)	-	-	-

S: sensitive, I: intermediate, R: resistant, Colistin**NCCLS1981*** : measures of National Committee for Clinical Laboratory Standards . Colistin**Gales** :** measures of **Gale et al, 2001.** Tigecycline*: for *Enterobacteriacae* use U.S FDA interpretative criteria, for *Acinetobacter spp* use interpretative criteria of Jones criteria.

Table.4 MIC distributions of Colistin and Tigecycline for tested isolates

Colistin					
species	**MIC_{50}** (µg/L)	**MIC_{90}** (µg/L)	**Range** (µg/L)	**S**	**R**
K.pneumonae(11)	0.5	16	0.5-16	8 (72.7%)	3 (27.3%)
K.spp(11)	0.5	2	0.5-2	11 (100%)	0 (0%)
Enterobacter spp(20)	0.5	2	0.5-4	17 (85%)	3 (15%)
Total CRE (47)	0.5	2	0.5–16	38 (80.9%)	9 (19.1%)
Acinetobacter spp(30)	0.5	1.5	0.25-2	30 (100%)	0 (0%)
Tigecycline					
*K.pneumo*nae(11)	0.5	1	0.25-2	11 (100%)	0 (0%)
K.spp(11)	0.5	1	0.25-16	10 (90.9%)	1 (9.1%)
Enterobacter spp(20)	0.5	1	0.25-2	20 (100%)	0 (0%)
Total CRE (47)	0.5	1	0.25 -16	46 (97.9%)	1 (2.2%)
Acinetobacter spp(30)	2	4	0.5-16	18 (60%)	12 (40%)

S: Sensitive, R: resistant, CRE: carbapenam resistant *Enterobacteriacae*

A study conducted in US medical centers in 2005-2009 found sensitivity to TIG was 98.2% for CR *K.spp* (MIC(50/90), 0.5/1 μg/mL), 98.4% for *Enterobacter spp.* (MIC(50/90), 0.25/1 μg/mL;), however TIG sensitivity for CR *Acinetobacter spp* was 86.2% (MIC(50/90), 1/4 μg/mL) as it was tested by BMD method (Sader et al., 2011). Also Marchaim *et al.(*2014) reported sensitivity of TIG by E test 79.3 % for CRE, 40.3 % for *A. baumannii* and the only isolates that show differences in TIG MICs according to the *in vitro* testing methods were *A. baumannii* isolates with higher MIC levels by E test in comparison with other three methods (BMD, Vitek-2, MicroScan). But still E test widely used for determination of MIC due to its easiness and availability.

In the current study, TIG (sensitivity : 97.9 %, MIC90=1 μg/ml) was more active against CRE in comparison with COL (sensitivity : 80.9%, MIC90=2 μg/mL). Also in a study collected 104 of CRE distributed in medical centers worldwide, TIG sensitivity was (100%) in comparison with polymexin B (88.1%) (Castanheira et al., 2008). Regarding *Acinetobacter spp*, COL sensitivity (100%, MIC90=1.54μg/mL*)* was higher than TIG (sensitivity : 60%, MIC90= 4μg/mL). This was in agreement with a study in South Africa applied on 232 of CR *A.baumanii* isolates that were fully susceptible to COL, 78% to TIG and 76% to amikacin (Ahmed et al.,2012).

Since DD is the most common used method of antibiotic sensitivity in our hospitals due to its flexibility, low cost and unavailability of automated techniques like other countries (Peerayeh et al.,2014). So selection of appropriate criteria for DD method is mandatory. In the current study COL sensitivity of *Acinetobacter spp* decreased from (100%) by using NCCLS1981 measures to (70%) by application of Gales criteria and in *Enterobacteriacae* from (83%) to (19.1%) respectively. A study reported that all sensitive or intermediate results by DD method should be confirmed by MIC test, but a resistant result by DD is true (Maalej et al.,2011). So E test can be for COL susceptibility as a simple method with excellent concordance with the agar dilution (the reference method) (Lo-Ten-Foe et al., 2007, Maalej et al.,2011).

In the current study the sensitivity of *Acinetobacter spp* to TIG decreased from (43.3%) by Jones criteria to (13.3%) by U.S.FDA criteria. Since no interpretive criteria have been approved for TIG against *Acinetobacter spp*, some studies use interpretative data of *Enterobacteriaceae* and found also increase resistance of *Acinetobacter spp* to TIG from (4.2%, 8.8%) by using Jones criteria to (20.8%,17.6%) respectively by U.S.FDA criteria (Peerayeh et al.,2014). Liu *et al.*(2012) proved good correlation between DD and BMD methods using both FDA and EUCAST interpretive criteria in testing sensitivity of TIG to MRSA,VRE, and ESBL-producing *E. coli* isolates but recommend the used of BMD not the DD method when testing ESBL-producing *K. pneumoniae* and *A. baumannii*.

In conclusion study of antibiotic susceptibility pattern of MDR bacteria is crucial in selection of appropriate antimicrobial therapy. In this study COL and TIG continue to be active against CRE and CR *Acinetobacter spp*. With reported resistance of COL among CRE and TIG resistance among *Acinetobacter spp*. So further studies are mandatory to discover new drugs that cover these widely spread MDR organisms.

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