Co-infection of Covid -19 patient with *A. baumnnii* in Iraqi patients

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Abstract

Acinetobacters.baumannii is an opportunistics pathogenshave a highs incidences through immunocompromised individuals, especially those who have experienced elongated hospital stay. To investigate the prevalence of antibiotics resistance genes of *A. baumannii* in Iraqi Covid -19 patients. Fifty clinical isolats of out of (86) isolate of *A. baumannii* were isolated patients attacked with Covid-19 from different clinical sources and from different hopsitals. All the clinical samples primarily were cultured on Blood, MacConkey agar. For differentiation *A. baumannii,* the clinical samples were also streaked on Drigalski Lactose agar medium. Suspected colonies were confirmed by VITEK-2 system. Co infected *A. baumannii* sample with Covid -19 were subjected to molecular detection with 16srRNA and bla NDM-2 gene. Results of this study found that (50) isolate of *A. baumannii* with multidrug resistant (MDR) and (XDR) proerties out of (86) isolate of *A. baumannii* were diagnosed molecularly by 16srRNA and identify 32 (64%) positive sample out of (50) isolates. Also results observed that the 21 (65.6%) sample out of 32 (positively sample for 16sRNA) have COVID-19 infection. Coinfected patients were more in male than female groups (76.2% , 23.8%) respectively. Also result found that antibiotic resistant gene (*bla*NDM-2) for *A. baumannii* associated with covid-2 were 15 (71.4%) in patient with covid -19. In conlusion the patients attacked with both Covid-19 and *A. bumannii* are at high risk of longer stay in hospital and potentially death also this study concluded the prevalence of antibiotic resistance among co-infected pateints especially with amikacin and tetracyclin antibiotics.

Keywords: *A. baumnnii, COVID-19, blaNDM-2, 16 SrRNA .*

دراسة عدوى COVID-19 المصاحبة للاصابة بالراكدة البومانية لبعض المرضى العراقيين

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الخلاصة

A. baumannii الراكدة البومانية من مسببات الأمراض الانتهازية ، نسبتها عالية في الأفراد الذين يعانون من نقص المناعة، وخاصة أولئك الذين عانوا من الإقامة الطويلة في المستشفى. ولمعرفة انتشار جينات مقاومة للمضادات الحيوية لبكتريا A. baumannii لمرضى كوفيد -19 العراقيين. تم عزل خمسين عينة سريرية من أصل (86) عزلة من A. baumannii من المرضى المصابين بفيروس Covid-19 من مصادر سريرية مختلفة ومن مختلف المستشفيات. تم استنبات جميع العينات السريرية بشكل

أساسي على أجار الدم ، MacConkey ، من أجل تمييز *A. baumannii* ، تم تخطيطها على وسط Drigalski Lactose agar. وتم التاكد من المستعمرات المشتبه بها بواسطة نظام VITEK-2. تم إخضاع عينات *A. baumannii* من المرضى المصابين بـ Covid- 19 للكشف الجزيئي باستخدام جين 16sRNA و blaNDM-2. أظهرت نتائج هذه الدراسة أنه تم تشخيص (50) عزلة من *A. baumannii* ذات المقاومة للأدوية المتعددة (MDR) و (XDR) من أصل (86) عزلة من *A. baumannii* بواسطة 16 sRNA وتم تشخيص 32 (64%) عينة إيجابية من أصل (50) عزلة. كما لاحظت النتائج أن 21 (65.6%) عينة من 32 (عينة إيجابية لـ 16sRNA) مصابون بعدوى covid- 19. كان المرضى المصابون بالعدوى المصاحبة أكثر في الذكور مما في النساء (76.2% ، 23.8% على التوالي). أيضًا وجدت النتيجة أن الجين المقاوم للمضادات الحيوية(blaNDM-2) لـ *A. baumannii* المرتبط بـ covid-2 كان 15 (71.4%) في المرضى المصابين بـ 19 – covid. المرضى المصابين بعدوى الراكدة البومانية وعدوى فايروس كورونا سويا يكون معرضين لخطر الرقود في المستشفيات لفترة طويلة وكذلك معرضين لخطر الموت، نتائج هذه الدراسة اوضحت انتشار مقاومة بكتريا الراكدة في الاشخاص المصابين بفايروس كورونا للمضادات الحياتية وخصوصا مضاد التتراسايكلين والاميكاسين

الكلمات المفتاحية : الرنا الريباسي16S الراكدة البومانية ،جين بلانديم، فايروس كورونا .

Introductions

A.baumannii is a grams-negative (G-ve), non-lactose fermenting organism that was increasingly recognized as a major pathogens causing nosocomials infections including ventilator-associated pneumonia, bacteremia,urinary tracts infection, meningitis and wound infections particularly in patients admitted to intensives care units (ICU) [1]. *Acinetobacte. baumannii* can alsoe cause community-acquireds infections, which are encountereds mainly in tropicals Australia and Southeast Asias [2]. For Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), hospitals-acquired infections(HAI) was defined as secondary infection occurring more than 48 hours after hospitalization.Pneumoniase occurrings 48 hours or more after hospitalization or endotracheal intubations were defined as hospitals-acquirede pneumonia (HAP) and ventilators-associated pneumoniae(VAP) [3]. (SARSCoV-2) infection can produce severesand critical respiratory failure required septic shock,mechanical ventilation, or others organ dysfunction or losss necessitates intensives care treatment [4]. The carbapenems resistance occurred in *A. baumanniie* shortly later the introduction and therapeutic use of carbapenems and disseminated globally, thermanagement of patientse with coronavirus disease 2019 (COVIDs-19) will be greatly problematic especiallys when co-infecteds with antibiotic resistants bacteria commonly distributede in hospitals [5]. During the COVIDs-19 pandemics, co-infection with *A. baumanniie* secondarysof SARSCoV-2 infections occurs. An isolates with (OXAe-23) genes has been responsibles for an outbreaks in COVIDs-19 ICUs of a tertiarys Japanese hospital [6]. Incidences of secondary infectionse(mostly lowers respiratorys tracts infectionse) attributeds to *A. baumanniie* hase been reporteds to be as high as (1%) of hospitalizeds COVIDs-19 patients in an Italians hospital (6).A

recents studys from a Frenchs intensives care treatment (ICU) calculated co-infection with *A. baumannii* at (1.1%) (1 outsof 92; susceptibles to third generations cephalosporinse) in severesSARS- CoV-2 pneumoniaspatients [7].

Recently, the *bla*NDM-2 variants (Pro to Ala substitutions at positions-28) was described, this alleles was firsts founds in a multidrugs-resistant (MDR). *A. baumannii* strain isolated from a German patient having previously beens hospitalized in Egypt.While a subsequents one was isolated in Israel [8]. The presences of super-infections in the lowers respiratory tracts of patients with COVID-19 due to that *A. baumannii* area resistantsto the extended-spectrumsantibiotics commonlys used for the treatments of life-threatening bacterial diseases, especially in ICU patients. Secondarys bacterial infections may develops during or following COVIDs-19 and thus they areaan undeniable fact, as a result of severes pandemics conditions, it wase not possible to have a negative controls groups without COVIDs-19 ine ours ICUs simultaneouslys [9]. Due to the previous information so this study was aim to, detection the Co-infection of *A. baumannii* with COVID-19 infections and related of antibiotic resistant gene in these patients.

Material and method

Bacterial identification

Fifty isolate *A .baumannii* out of (86) patients with COVID-19 patients in both sexes. The collected clinical sample from different patient's sources (burns, sputum, wound swabs) were subjected for streaking methods directly on Blood agar and MacConkey agar. Further identification by morphological characteristics, biochemical, and VITEK-2 System. By using bacterial suspension then The inoculated card has been passed through a mechanism carousel incubator according to [10].

Antibiotic resistance

Antibiotic sensitivity of *A.baumannii* isolates by Kirby-Bauer disk diffusion method were applied to study the effect of seven type of antibiotic including: Amikacin, Ceftriaxone, Imipenem, Levofloxacin, Meropenem, Polymyxin-B and Tetracycline (Bioanalyse- Turkey).This test were done by transferring a sufficient amount from a pure culture of the isolates into a tube of normal saline solution with optimum concentration then streaking over the surface of the (MHA)Muellers-Hinton Agars plates. Afters 24 hours of incubations at 37 ℃, the inhibition zone diameter was measured, and the results were classified as sensitive, intermediate, or resistant [11].

Molecular detection

DNA of bacterial *A. baumannii* used in study were extracted by (Gram-negative bacteria) according to Kits WizPrep™ DNA Mini Kit [12]. After measurement the concentration and purity of

the DNA mixed with primer for 16sRNA [13] , and desgin for bla - NDM primer (F : AACAATTGTCAGAAGGGCGG ; R: TAGCAACAGGTTCCGGTTCT). Genomic DNA of *Acinetobacter baumannii* was used to detect the *16SrRNA, blaNDM-2* genes and that have been performed through the conventional PCR with 25µl of the PCR Master mix reaction.

Statisticals analysises

Datas of currents study were analyzed by usings Chi-squares (X^2) test to compared between percentagess. A levels of significance of $\alpha=0.05$ was applieds to tests. (SPSS v.23 programss used to analyze currents data.

Results and Discussions

Coronasevirus diseasess2019 (COVID-19) is a recent form of respiratory problem as consequen of (SARS-CoV-2) with very broad clinical spectrum. All the sample was culture on MacConkey agar, blood agar, then diagnosis were confirmed by biochemical test with catalase positive, oxidase negative for. *A. baumannii* grow on Drigalski Lactose Agar [14]. Also result insure that the isoleted sample were *A. paumannii* with VITEK-2 system according to [15] . The results of the current study observed that the 21 (65.6%) positive covid 19 sample from 32 (16SrRNA positive) of *A. bumannii* infection. While *A. baumannii* wase identified in (20%) ofe sample acquireds from COVIDs-19e patientse in an (ICU) in Beijings, China, durings lates(ICU) admission [16]. Compared co-infections in criticallys ills patients with or withoutsCOVID-19, also *A. baumanniie* was the mosts recurrently isolateds bacteriums among patients admitted to intensive care units (ICUs) with different periods. Result show the coinfected patients were more in male than female groups 16/21, 5/21 (76.2%, 23.8%) respectively (Figure 1). Thes results of this study were agreement with results of (5), who docuemeted that (COVIDs-19) patientse were diagnosed with Carbabenem-resistance *A. baumannii* (CRABs), co-infection were (66.7%) male and female (33.3%).While a study in an Iranian of COVIDs-19 patients, (58%) patients weres males and (42%i) were females, with a means age of (~ 67)years olds, (89.5%) of Covids-19 patients weres co-infected through strains of *A. baumannii,* the rest (10.5%) cases were reported as *S. aureus* [17].
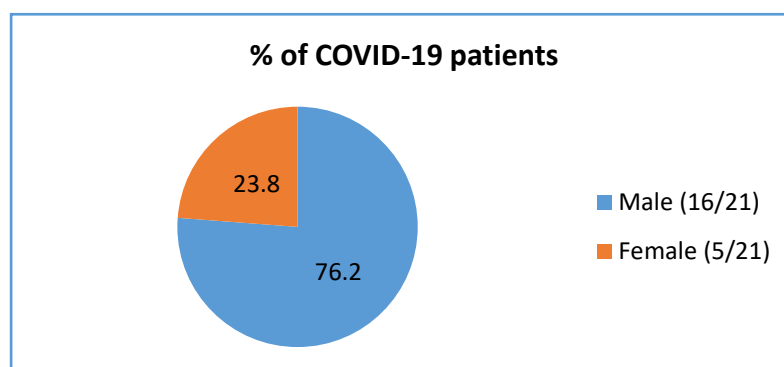
Fig. (1): Distribution *of A. baumannii* infection in covid -19 patients according to gender groups.

Current study was declared that (50) *A. baumannii* isolated with MDR& XDR properties from different source in , there were 21(65.6%) isolates from sputum in COVİD-19 patients out of positive isolate of 16sRNA gene, burns with percentage 19(38%), while wound isolate 5(10 %) that show in (Table-1). This result declared that the highest percentage of isolate from sputum in covid-19 patients. While not found any isolate from burn or wound in covid-19 patients with non significant differences.

Table (1): Distribution *of A. bumanii* infection according to genders and COVID-19 infection status and type of clinical samples.

Sample	COVID-19 status	Gender		Total	*p*-value
		Male (N=34)	Female (N=16)		
Sputum	COVID-19 -Positive	16 (76.2)	5 (23.8)	21 (100.0%)	0.8 (N.S)
	COVID-19- Negative	3 (60.0%)	2 (40.0%)	5 (100.0%)	
Burn	COVID-19- Negative	12 (63.2%)	7 (36.8%)	19 (100.0%)	-
Wound	COVID-19- Negative	3 (60.0%)	2 (40.0%)	5 (100.0%)	-
Total				**50 (100.0%)**	

Through the COVIDs-19 pandemic, co-infection with *A. baumannii* secondarys to SARSCoV-2 infectionse has beensreported. The studied focusing on co bacterial infection among COVID-19 cases, (16.6%) *A. baumannii* bacterial isolates were obtained from respiratory samples [18].

Antimicrobial susceptibility tests were done for all *A. baumannii* isolates from covid 19 patients (21 isolates) by using the disk diffusion method (Kirby-Bauer Method) against (7) different clinically important antimicrobials. These isolates showed different susceptibility toward antimicrobials used in this study. The susceptibility to different antimicrobials was determined depending on CLSI (2020) [19].

The result of antibiotic sensitive declared that high antibiotic resistance in Covid 19 patient co-infected with *A. baumannii* with highest rates for amikacin (95.23%), while the percentage of tetracycline resistance (90.47%).

Addionaly ceftriaxone is a third-generation cephalosporin class use to treatsinfections produced by(G-ve)Gram negative bacilli [20].This study showed that the resistance rate against ceftriaxone was (71.42.0%).

Also, CRAbs infectionse in COVIDs-19-positivee patients admitteds to the ICU were morea commons compareds to those thatswere COVIDs-19 negativea(21). During the COVIDs-19 pandemics, co-infection withs *A. baumanniia* secondarys to SARSCoV-2 infectionse hase beens reporteds. Among the studied focusing on co-bacterial infection in COVID-19 cases. Result revealed that *A. baumannii* isolates had moderate resistance to Meropenem with percentage (57.1%), while the resistance to Imipenem and   Levofloxacin were (23.8%). The lower percentage of resistance was recorded to polymyxin- B (9.52%). (Figure -2).
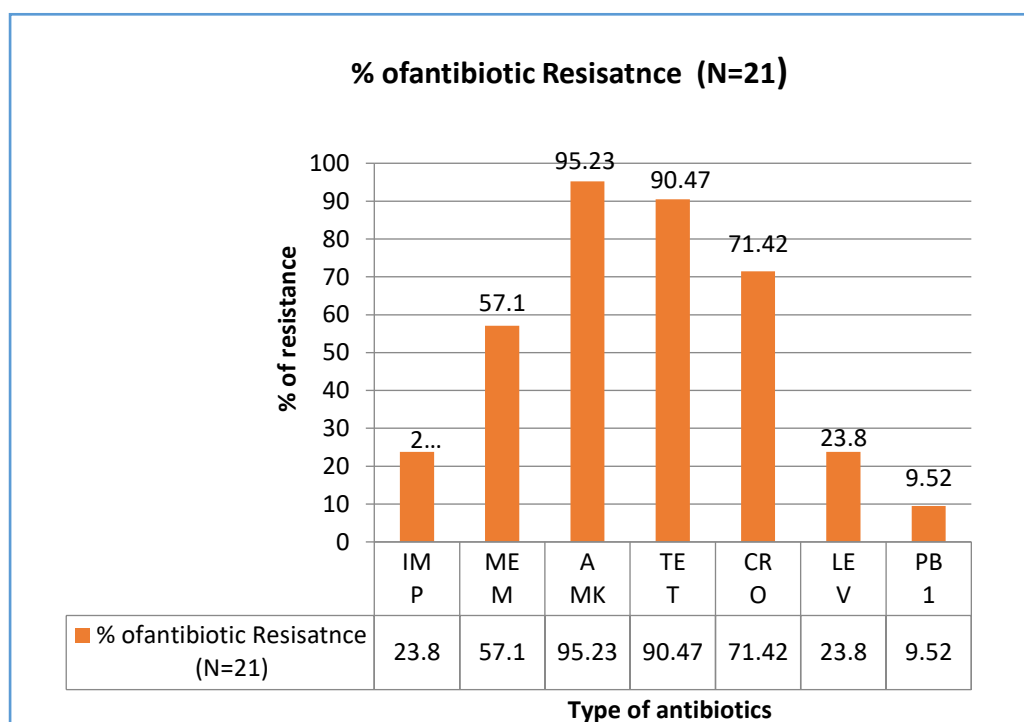

% ofantibiotic Resisatnce (N=21)

	IMP	MEM	AMK	TET	CRO	LEV	PB1
% ofantibiotic Resisatnce (N=21)	23.8	57.1	95.23	90.47	71.42	23.8	9.52

Fig. (2): Antibiotic susceptibility of A. baumannii strains (N=21) associated with covid-19 patients. IMP10(Imipenem), MEM10 (Meropenem), AK 30(Amikacin), TET 10 (Tetracycline) LEV 5 (Levofloxacin), (CTR 30) ceftriaxone, PB 300(polymyxin-(B).

Result of [21] sample suffer from covid-19 out of 32 (64%) that have MDR and XDR which have positive 16srRNA the diagnostic gene for *A. baumannii* and 18(36%)which have negative 16SrRNA in (Figure-3).
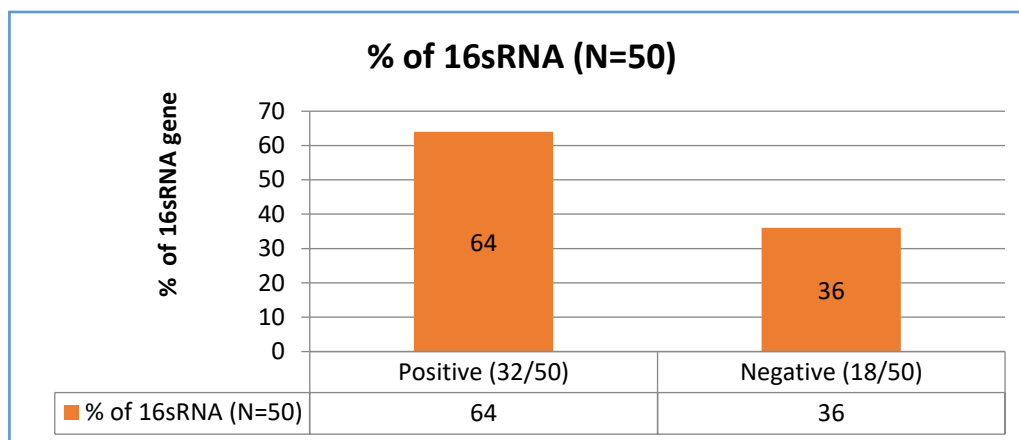

Fig. (3): Distribution of 16SrRNA the diagnostic gene for *A. baumannii.*

Result found that the presence of *bla*NDM-2, *Tet (B)* and *bla*TEM gene with percentage 15(71.4%), 12 (57.1%) and 10 (47.6%) respectively [22], The expressions of *bla* NDM lactamasese does nots compromises bacterial growths and is favoreds overs other (aMBLs) metallo beta lactamase duea to the lacke of fitness costs leadings to itse worldwides disseminations amongs (G-ve) Gram-negatives bacterias .
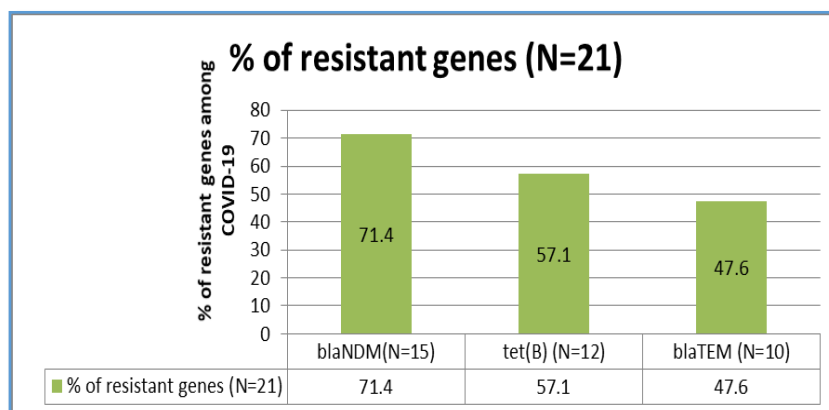

Fig. (4): Antibiotic resistant gene for *A. baumannii* associated with COVID-19.

While results of gene resistant to tetracycline was 57.1% this was in accordance with [23] who mention that the seconds categorys refers to tetracyclines majors facilitators superfamilys (MFSe) ,effluxs pumpse: *tet* (A) and *tet* (B). so the result declared that tet (B) gene was most common antibiotic in covid patient co-infected with *A. baumannii.* Theahighest occurrence of MDRs *A. baumannii* co- infection was documented in an Egyptian patientse hospitalizeds with COVIDs-19,

susceptibles only to tigecyclines andi fluoroquinolonese, havingi resistances genes *bla*NDM-1, *bla* TEMs, ands CTXi-M) [24]. Also the result found that the more common *bla* NDM-2 gene among co infected sample with percentage 15 (71.4) for positive isolate and 6 (28.6%) for negative isolate (Figure 5).
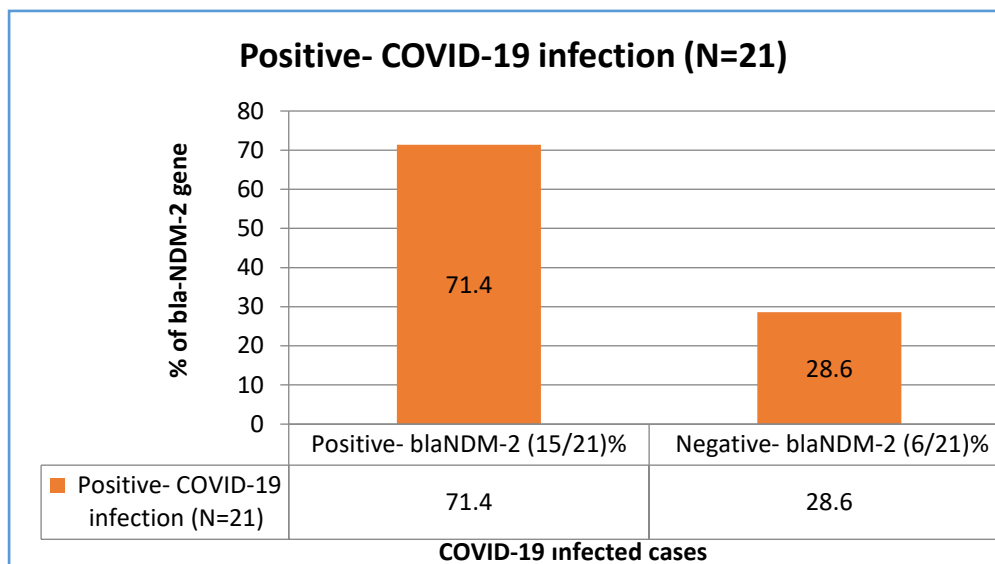

Fig. (5): Distribution of antibiotic resistant gene *bla*NDM of *A. baumannii* in infected cases with COVID-19.

According to the study results, the prevalence of the *blaNDM-* gene is higher than results obtained by [6،25] who suggested the most powerful colonization of *A. baumannii* in patient with Covid 19.While the result of antibiotic sensitive declared that high antibiotic resistance in COVID-19 patient Co-infected with *A. baumannii* to Amikacin,and the most antibiotic effect on the bacteria Levofloxacin and Imipenem and finally the Polymyxin-B. Thea expressions of *bla*NDM lactamasese does not compromises bacterial growths and is favored over others (MBLs) due to the absence of fitnesse costs leadings to its worldwides disseminations amongs(G-ve) Grams-negative bacteria in [22].

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