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MOLECULAR STUDY OF *Acinetobacter baumannii* USING *16S RRNA* AND *BLA OXA-51* GENE ISOLATED FROM HOSPITALS IN DUHOK-KURDISTAN REGION, IRAQ

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ABSTRACT

Background and objective: *Acinetobacter baumannii* is pathogenic and multiresistant bacteria that cause nosocomial infection. The aim of this study are diagnosis A. *baumannii* by using specific bla OXA-51 and 16SrRNA.

Methods: out of 150 *Acinetobacter species* samples were collected from patients in Azadi teaching hospital, Duhok Emergency hospital; and burn Duhok cosmetic and burn hospital from May 2020 to August 2020. The samples were phenotypically diagnosed by bacteriological strategy. Out of 100 samples were selected and by molecular level confirmed as *Acinetobacter* spp.

Results: isolates were revealed as small, pale, and late-lactose fermenter colonies on MacConkey agar appeared as a creamy, opaque, andnon-hemolytic colony on blood agar All suspected isolates, as A. baumannii, were growing at 44ºC. By using the genus-specific bla OXA-51 primer that produced 353 bp amplification band and 75 samples were diagnosed by PCR as *A. baumannii by* 16S rRNA as a specific primer and showed 150 bp amplicon. 10 samples out of the 75 resembling *A. baumannii* were identified as a multidrug resistant isolates by method of diffusion. Disc, *A.* baumannii isolates displayed a high resistance rate of 85% to azithromycin and 80% to imipenem. Moreover, amikacin, meropenem, and gentamycin, Trimethoprim-Sulfamethoxazole, and ciprofloxacin, norfloxacin showed a low level of efficacy against A. baumannii isolates with the resistant rate of 88%, 90%, 90%, and 93% respectively.

KEYWORD: Acinetobacter baumannii, bla OXA-51, multi-drug resistant, 16S rRNA, PCR

1. INTRODUCTION

Acinetobacter is currently classified under the family *Moraxellaceae*, which includes the genera *Moraxella, Psychrobacter*, and other related organisms (Almasaudi., 2018). *Acinetobacter baumannii* (*A. baumannii*) is considered as an opportunistic pathogen causing nosocomial infections in hospitalized patients, particularly in Intensive care units (ICU), as well as community-acquired infections (Jessie *et al.*, 2020). These infections include pneumonia, bloodstream infection, skin and soft tissue infections, wound infection, meningitis, urinary tract infection and endocarditis (Liu *et al.*, 2017). *Acinetobacter* infections have been historically associated with military and injured soldiers in combat due to direct environmental contamination of wounds (Diancourt *et al.*, 2010). During the past decade, the incidence of nosocomial outbreaks by *A. baumannii* has been described mostly in burn, surgical and intensive care units (Jafari and Karbasizade, 2014). Most surveillance studies report high mortality rates among patients with *A. baumannii* bacteremia and may be associated with considerable morbidity and mortality reach up to 70% (Wong *et al.*, 2017). The management and prevention of *A. baumannii* spreading health care settings require identifying potential reservoirs of this organism, the modes of transmission and investigating *A. baumannii* clones which cause the outbreaks from epidemiologically unrelated strains. A comparison of isolates at the subspecies level is required by the application of molecular typing methods (Sadeghi *et al.*, 2016). The use of molecular tools, especially the Polymerase Chain Reaction (PCR), has a great impact on simplifying and specificity for identification, characterization, and taxonomy of infectious agents (Shahi *et al.*, 2018).

2- MATERIALS AND METHODS

Out of 150 samples were collected from burns and sputum inpatients in Azadi teaching hospital, Duhok Emergency hospital; and burn Duhok cosmetic and burn hospital between

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May-August 2020 both genders bunches. The tests were managed according to the standard bacteriological strategy including; growth on MacConkey agar, blood agar, catalase and oxidase tests, (McFaddin, 2000). All clinical samples were undergoing to DNA extraction tests by using the genomic DNA purification kits (Bioneer®, Korea); DNA results was showed by light of UV and then electrophoresed on agarose gel. 1 % (Ghaima et al., 2016). PCR was done by adding 4 ml of the genomic of bacteria to 12.5 ml mastermix (Merk KGaA PCR premix(Germany)), and also add 1.2 ml (20 picomolμl) of the bla OXA-51 primer (Ghaima *et al.*, 2016). the final volume was completed by adding 6.1 ml Dionized distilled water. The amplification sizes groups and primers sequences of bla OXA-51 and 16 rRNA show in table 1 PCR conditions for increasing bla OXA-51 quality is outlined in table2.

Table 1: molecular weight and Primers sequences of PCR products genes used

Gene	Forward primer	Reverse primer	Product size(bp)
OXA-51	5-TAATGCTTTGATCGGCCTTG-3	5-TGGATTGCACTTCATCTT GG-3	353
16 rRNA	5-CAGCTCGTGTCGTGAGATGT-3	5′-CGTAAGGGCCATGATGACTT-3	150

Table 2: PCR-amplification and cycle condition of *bla OXA-51* gene.

Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
94C°	94C°	52C^0	72C°	72C°
5min	45seconds	40seconds	45seconds	6min
1 cycle		30 cycle		1 cycle

16 sRNA primers in table 3. the running conditions for the amplification of 16 sRNA of *A. baumannii* gene showed in table 3, PCR product tests were run on gel electrophoresis on 1 % agarose and the DNA bands were showed under light of UV and shot by Camera (Ghaima *et al.*, 2016). Anti-microbial sensitivity profile test of *A. baumannii* samples continued by the method of Diffusion Disc strategy on Mueller-Hinton agar, and a group of antimicrobs was chosen according type of Bacteria. Strategy entirely used by (Ghasemi *et al.*, 2018).

Table (3): PCR-amplification and cycle condition of 16 sRNA gene.

Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
95C°	95C°	55C	72C°	72C°
3min	1min	1min	1min	5min
1 cycle		30cycles		1 cycle

RESULTS

all the enlisted 150 clinical sample were showed phenotypic characteristics of *Acinetobacter spp*. isolates were revealed as small, pale, and late-lactose fermenter colonies on MacConkey agar appeared as a creamy, opaque, andnon-hemolytic colony on blood agar All suspected isolates, as *A. baumannii*, were growing at 44ºC, which indicates the ability of these bacteria to grow at the high-temperature degree (Belay *et al.*, 2018). The samples disclosed the presence of genomic of bacterial DNA after extraction method. Results moreover affirmed that arbitrarily chosen 100 samples had been distinguished as *Acinetobacter spp* at the molecular level. (Figure 1)

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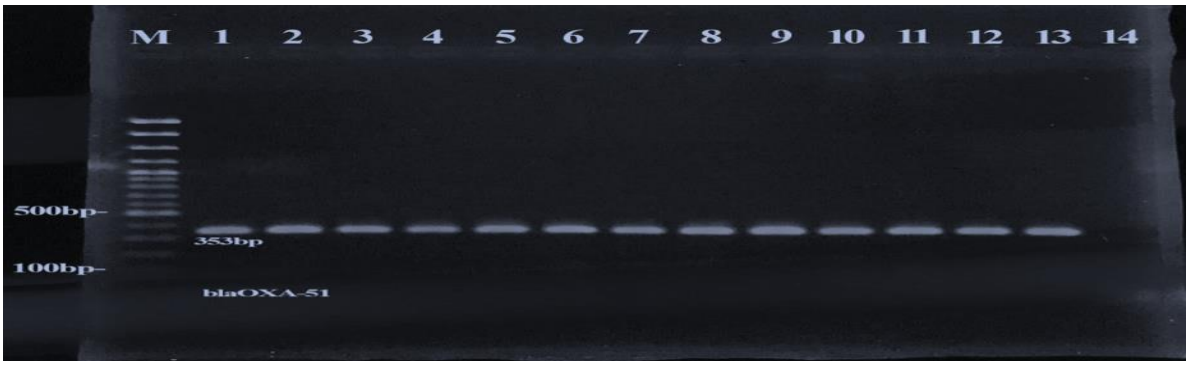

Fig. (1): *Acinetobacter spp*. strains PCR amplification produced with bla OXA-51 gene with M.W. 353bp. Lane M is DNA lader (1500-100bp).

For the molecular recognizable proof of *A. baumannii*, primer was utilized to distinguish target 16 S rRNA, and the result appears that 75 out of the arbitrarily chosen 100 samples in 150 bp species-specific amplicon Figure 2. The results of the antibiotic sensitivity test by the Kirby Bauer method are shown in Table 4. Colistin was considered as one of the most powerful antimicrobial agents, where none of these isolates were resistant to this antibiotic. Doxycycline with a resistance rate of 39% may be considered as the second most effective antimicrobial against *A. baumannii* isolates. Similarly, levofloxacin had a moderate effect on *A. baumannii* isolates with a 51% resistant rate. Indeed, *A. baumannii* isolates displayed a high resistance rate of 85% to azithromycin and 80% to imipenem. Moreover, amikacin, meropenem, and gentamycin, Trimethoprim-Sulfamethoxazole, and ciprofloxacin, norfloxacin showed a low level of efficacy against *A. baumannii* isolates with the resistant rate of 88%, 90%, 90%, and 93% respectively. Our results revealed that 10 *A. baumannii* isolates (24.4%) were found to be multidrug-resistant (MDR) and 27 isolates (65.9%) were XDR. Determination of MDR and XDR among isolates of *A. baumannii* was done according (Coskun *et al.*, 2019) who define MDR as a resistance to at least one agentin three or more classes of antimicrobial agents, while XDR is resistance to at least one agent in all classes but remained sensitive to only one or two antimicrobial agents (Coskun *et al.*, 2019).

Table (4): Antibiotic resistance profile

Antibiotics	Code	Sensitivity		Resistance	
		No	%	No	%
Amikacin	**AK**	5	12%	36	88%
Gentamycin	**CN**	4	10%	37	90%
Ciprofloxacin	**CIP**	2	5%	39	95%
Levofloxacin	**LEV**	18	44%	23	56%
Norfloxacin	**NOR**	1	2%	40	98%
Doxycyclin	**DO**	23	56%	18	44%
Trimethoprim-Sulfamethoxazole	**SXT**	3	7%	38	93%
Azithromycin	**AZM**	6	15%	35	85%
Imipenem	**IPM**	8	20%	33	80%
Meropenem	**MEM**	4	10%	37	90%
Colstin	**CT**	41	100%	0	0%

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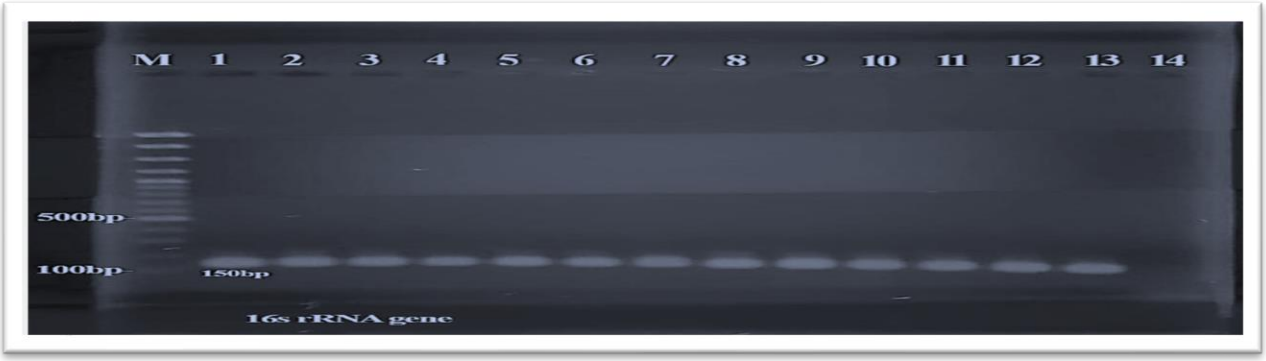

Fig. (2): Bands of PCR product for 16 S rRNA of *A. baumannii* on 1 % agarose gel.
Lane M is DNA ladder (1500-100bp).

DISCUSSION

A. baumannii strains is increasing, particularly affecting patients who are immunocompromised and hospitalized in intensive care and burns units (Sepideh *et al.*, 2020). *A. baumannii* could be a significantly advanced nosocomial microorganism that's regularly experienced in clinic settings in portion due to the capacity of this bacterium is to survive on dry or moist surfaces, on healthy skin and may be present in foodstuffs, in addition to its ability, to recruit different intrinsic and acquired mechanisms to resist different antibiotics (Abbott *et al.*, 2013). *A. baumannii* moreover improvements of its chromosomal DNA which allow phenotypic transformation and in this way increment the potential for creating antimicrobial resistance and the capability to outlive in sea-going conditions on restricted supplements supply (Zarrilli *et al.*, 2009). An *OXA-51*-like gene is the largest group which belongs to the Ambler Class D oxacillinase group (OXA-type). There are 95 enzymes variants of this group that have been identified to date (Evans and Amyes, 2014). These enzymes are intrinsic and naturally exist in the chromosomal DNA of *A. baumannii* inherited into all strains (Shahi *et al.*, 2018). This fact makes it a wonderful marker successfully applied for the detection of *A. baumannii* at the species level (Shahi *et al.*, 2018; Ghaima *et al.*, 2016). Although all these enzymes play an important role in resistance against carbapenems, only two of the *OXA-51*-like enzymes, including; *OXA-51* and *OXA-69* showed weak hydrolytic activity toward the carbapenems (Zarrilli *et al.*, 2009). Therefore, the *OXA-51* gene presents among all *A. baumannii* strains. It is worth to mention that the insertion of the IS element *A. baumannii* 1 (IS*Aba1)* upstream of the *OXA-51* gene may change the expression of this gene which results in resistance to board spectrum antibiotics (Esther *et al.*, 2013).*16S rRNA* gene used as a marker for the identification of different bacteria at the genus level because it is more powerful, reproducible and precise than those results obtained via conventional methods (Belay *et al.*, 2018). using 16S rRNA sequences, it is possible to study bacterial taxonomy and phylogeny. So far, it is the most common genetic marker used for a number of reasons. It is present in almost all bacteria, often presenting in operons or multigene family and has consensus sequence with the function of the 16S rRNA region which has not changed over the time (Susanna *et al.*, 2015).

According to the antibiotic sensitivity profile, the high risk of these isolates has been identified due to exposing a high resistance rate against most of the used antibiotics. The results of antibiotics in this study are recorded according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2016). Colistin considered antimicrobial agent that has an effect on all isolates with a 100% sensitivity rate (Table 4). This result was agreed with results reported in Turkey by (Coskun *et al.*, 2019) who found none of *A. baumanni* isolates were resistant to colistin. Notwithstanding, different researches have been recorded the emergence of *A. baumannii* strains that developed resistance against colistin during treatment and subsequently responsible for disastrous outbreaks (Diancourt *et al.*, 2010). The emergence of *A. baumanni* isolates is resistant to colistin which has been reported in other studies conducted in Baghdad hospitals by (Ghaima *et al.*, 2016) who recorded these isolates in resistant rate 37.5% Colistin is a polypeptide antibiotic exposed high effectiveness against carbapenem-resistant Gram-negative bacteria which was usually

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intravenously administrated with high dose (Bergen *et al.*, 2012). Doxycyclin considered as the second most active antimicrobial agent with a resistance rate of 44%. Somehow, this result agreed with the obtained results by (Hussein *et al.*, 2013) who recorded 45%. However, the wide dissemination of ribosomal protection proteins and efflux pumps in *A. baumannii* protect it from these antibiotics and limit their therapeutic use (Doi *et al.*, 2015). Regarding their resistance to quinolones (levofloxacin and norfloxacin) showed with varied rates ranged from 56% to 98%, respectively. Levofloxacin was the most effective antibiotic among the quinolones group. It had a moderate effect on *A. baumannii* isolates. On the other hand, ciprofloxacin and norfloxacin were low effective against these isolates. This results were agreed with (Balaky *et al.*, 2019) in Western Emergency Hospital-Erbil, who showed that these isolates exhibited a high resistant rate (95% and 61%) to ciprofloxacin and levofloxacin, respectively. these isolates showed highly none susceptibility to Trimethoprim-Sulfamethoxazole with the rate of 93%. This antibiotic is a synergistic effect through a combination of TMP-SMX drugs to inhibit bacterial DNA synthesis (Karageorgopoulos and Falagas,2008). Regarding the ability of the isolates to resistant to aminoglycosides (amikacin, gentamicin) and (azithromycin), they exhibited a high resistant rates which were 88%, 90%, and 85%, respectively, and it were agree with another results recorded A. *baumannii* strains with a high resistant rate (69.6 % and 88.7 %) to Amikacin and Gentamycin respectively (Shali *et al.*, 2012). It's worthy to mention that gentamycin and amikacin are the most recommended options for the treatment of infections caused by *A. baumannii* (Clinical and Laboratory Standards Institute, 2016). Generaly, it has been found that tobramycin and amikacin exhibited a more potent effect than to gentamycin (Fishbain and Peleg, 2010). The usage of aminoglycosides in an intravenous administration as well as aerosol form approved high effectiveness for patients with cystic fibrosis and shows potential results among VAP patients (Badawy *et al.*, 2020). The results of this study also showed a high resistant pattern to Meropenem and Imipenem with a resistance rate of 90% and 80% respectively. In many years, the carbapenems were considered as the last instance for treatments of different infectious diseases (Abbott *et al.*, 2013). However, the resistance of *A. baumannii* to expanded-spectrum cephalosporins and carbapenems is rapidly growing over the years and limits therapeutic options, relying on polymyxins in combinations with other antibiotics (Chang *et al.*, 2015). The survival and existence of MDR *A. baumanni* in environmental hospitals may be the main cause of nosocomial infections worldwide (Ghasemi *et al.*,2018). Many researches in other countries including Iran and Turkey, have recorded a high percentage of MDR as well as XDR among *A. baumannii* strains (Heidari *et al.*, 2018). The emergence of MDR *A. baumannii*is largely associated with high mortality among hospitalized patients (Kanafani *et al.*, 2013). Furthermore, the emergence of XDR *A baumannii* strains may alarm the complication of treatment due to the ability of these isolates to resistant to almost all antibiotics but colistin.

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