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Detection of Bla_{oxa-23}andbla_{oxa-51} Genes in MDR *Acinetobacterbaumannii*isolated from Different Clinical Sources in Baquba Teaching Hospital in Diyala City, Iraq

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Abstract.*Acinetobacterbaumannii* is a nosocomial pathogenic bacteria that is increasingly important also mainly affects ill people. In the present research, blaOXA-23 and blaOXA-51 genes have been observed in *A. baumannii* clinical MDR isolates. Methodology: From 22 *A. baumannii* isolates, separate clinical specimens were collected from (burns, wounds , blood, urine and fluids) at Baqubah Teaching Hospital. The isolates were biochemically identified and confirmed it by the compact device VITEK 2. The resistance of isolates against antibiotics was tested using the procedure of disc diffusion for all of the following antibiotics (Amikacin ,Ampicillin sulbactam ,Ceftazidme, Ceftraxon , Ciproflaxacin , Colistinsulphate , Doxycylin ,Impenem, Levoflaxacin , Meropenem, Polymyxin B,TrimetheprimSulfomethazol). In order to identify blaOXA-23 and blaOXA-51 genes, conventional polymerase chain reaction(PCR) was accomplished. Results: This study found that of 22 isolates 11(50%) isolated from burns, 5(22.7%) blood, 3(13.6%)wounds , 2(9%) urine and 1(4.5%) fluids. The current study showed that there was high resistance (100%) to antibiotics (Amikacin, Ciproflaxacin andLevoflaxacin) while the resistance of isolates toward antibiotics (Ampicillin sulbactam, Ceftraxon, Doxycylin, Empenem, Meropenem, Trimetheprimsulfomethazol) were (95%, 81%, 90%, 40% 86%, 90% and 77%), respectively. Whereas resistance to polymyxin B and colistin showed 0%. BlaOXA-51 was detected in 22 (100 %), while blaOXA-23 was detected in 18 isolates (81 %). Conclusion: The study showed a high prevalence of blaOXA-23 and blaOXA-51 genes among *A. baumannii* isolates which highlighted the importance of molecular study in order to find the appropriate management and to avoid their spreading during the hospital location

Keywords: *A. baumannii* , blaoxa23 ,blaoxa51 , Antibiotic

INTRODUCTION

Acinetobacterbaumannii is an opportunistic coccobacillus pathogen, a major source of external health-related infections (1). *A. baumannii* is strictly aerobic ,a non-fermentative, non-motile, non pigmented, catalase positive also oxidase-negative . (2). *Acinetobacterbaumannii* and other pathogenic *Acinetobacter* spp. are important sources of nosocomial infections (skin or soft tissue infections, pneumonia, bloodstream infection, , urinary tract infection , meningitis) (3) *Acinetobacterbaumannii* may acquire multiple antimicrobial resistance pathways, leading to multi-resistant phenotype in certain instances (4). Infections caused by the Multidrug-resistant bacteria are the suggested candidates for the treatment using carbapenem antibiotics (5). The drugs of choice for the treatment of serious nosocomial infections caused by A. baumannii are carbapenems (6).H owever, resistance to carbapenem is gradually becoming a great worry, mainly among the nosocomial strains belonging to (ESKAPE) group of pathogenic species (7) . CRAB produc of carbapenemases and is due to the carbapenemhydrolysing class D β-lactamases. The OXAtypecarbapenemases, which are belong to class D β-lactamases are classified into five subgroups. Four of them are acquired carbapenemases, which include (OXA- 23, OXA-24, OXA-58 , OXA-143) ,the OXA-51 only one is intrinsic to A. baumannii (8). The blaOXA-23 gene is one of the most prevalent β-lactamase genes on the genome (mostly on the plasmids) of carbapenem-resistant A. baumannii. Specific and rapid identification of A. baumannii and the strains harboring blaOXA-23 gene, will suggestion referential information on the therapeutic and control defenses for the nosocomial infections owing to the (CRAB).(1,9)

The purpose of this report to investigate the OXA-51 and OXA-23 among multidrug-resistant A. baumannii strains, isolated from different clinical specimens in diyalacity,Iraq .

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Methods

Collection of Specimens and identification of isolates :

During the period from September (2020) to March (2021) we collected 22isolates of *A. baumanniis* from clinical specimens of (burns, wounds, urine, blood also fluids) at Baquba Teaching Hospital, Bacteriology lab. The isolates were cultured on MacConkey agar (MA) and blood agar (Merck, Germany) incubated at 37°C in 24 hours. and identified by using biochemical tests like (sugar fermentation, motility, usage of citrate, urease, O/F, catalase, oxidase; growth potential of 37 °C also 42 °C) (10).The identification was confirmed by VITEK 2 automated a device (bioMérieux, France) .

Antimicrobial susceptibility profiles:

The antimicrobial Susceptibility testing was performed based on Kirby-Bauer disk diffusion method according to Clinical Laboratory Standard Institute guidelines (CLSI)(11). Against 12 antibiotic disk (Bioanalyse/TURKEY): (Amikacin (30 mg), ampicillin/sulbactam (20 mg), ceftazidime (10 mg), , Ceftraxon (10 mg) , Ciprofloxacin (5 mg), Colistinsulphate(25 mg), Doxycylin (30 mg), Imipenem (10 mg), Levoflaxacin (5 mg), Meropenem (30 mg) and Polymyxin B(300 mg) .

GenomicDNAExtraction :

ExtractionofDNA from 22 isolates *A. baumannii* and the concentration of DNA werecalculatedusing a commercialpurificationmethod (Genomic DNApurificationKit,promega, USA).

Detectionof *blaOXA-23alsoblaOXA-51*Bypolymerasechainreactionanalyze(PCR)

In order to investigate of bla_{OXA-23} and bla_{OXA-51} genes , The primers used in current study shown in"**TABLE 1**" 12.5 l Master mix (MgCl2, dNTPs, PCR buffer, also Taqpolymerase), 2 l DNA sample, 0.5 l per primary, and 9.5 l PCR reaction mixture (25 l). Cycler ThermalCyclertoamplifygenes(Bio-RadLaboratories).Initialdenaturation(94°Cfor5minutes),then30denaturation cycles (94°C for 30 seconds) and even annealing (54°C and 58C for 30 seconds for blaOXA-23 and blaOXA-51respectively) were used in the PCR. The extension time (72°C for 45 seconds), accompanied by the finalextension(72°Cfor7minutes)attheconclusionofthecycling(12,13).A1.5%agarosegelstainedwith ethidiumbromidewasusedtoseparatethePCRingredients(EtBr).Fortheimage,thegelwasvisualizedusingageldocumentation scheme (UVTECCambridge, UK).(12,13)

TABLE 1. The primersusedinthisresearch

Primers	Oligonucleotide Sequences (5′ - 3′)	Product Size, bp
***blaOXA-23* F:**	**5′-GATCGTTGGAGAACCAGA-3′**	**501**
***blaOXA-23* R:**	**3′-ATTTCTGACCGCATTTCCAT5′**	
***blaOXA-51* F:**	**5′-TAA TGC TTT GAT CGG CCT TG 3′**	**353**
***bla OXA-51* R:**	**5′-TGG ATT GCA CTT CAT CTT GG 3′**	

Results

Susceptibility ToAntibioticsprofiles

Resistanceto12antimicrobialagentswasfoundamong high percent of A.*baumannii*isolates.Resistance to

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(amikacin, Ciproflaxacin, and Levoflaxacin) was 100%, while resistance to (Ampicillinsulbactam, Ceftazidme, Ceftraxon, Doxycylin, Impenem, Meropenem, Trimetheprim and Sulfomethazol) were 95 %, 81 %, 90 % , 40 % , 86 % , 90 % , 77 %) respectively . Colistinand PolymyxinB showed highest percent of sensitivity (100%) in all isolates as shown in (Fig. 1).

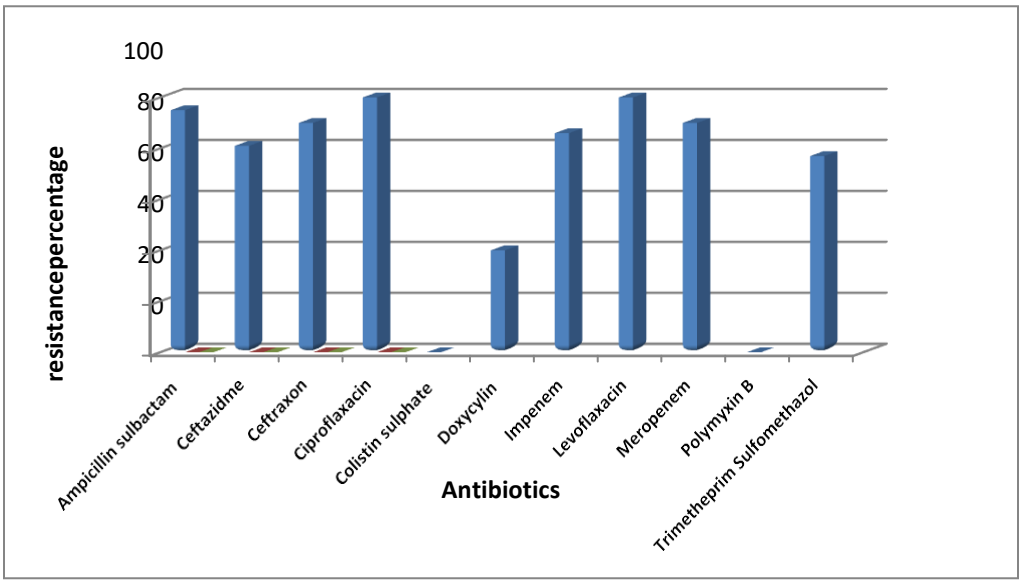

FIGURE 1: The resistancepercentageofMDR*A.baumannii*to12 antibiotics

Detection of bla$_{OXA-23}$ also bla$_{OXA-51}$ in A.buammanii using polymerase chain reaction (PCR):

Amplification of $_{blaOXA-51}$ gene was found in all 22 (100%) A.baumanniiisolatesafteranalysisof thePCR product.Whereasebla$_{OXA-23}$ genewas shownto beexpressedin18(81%)oftheparticipants "fig2, 3"

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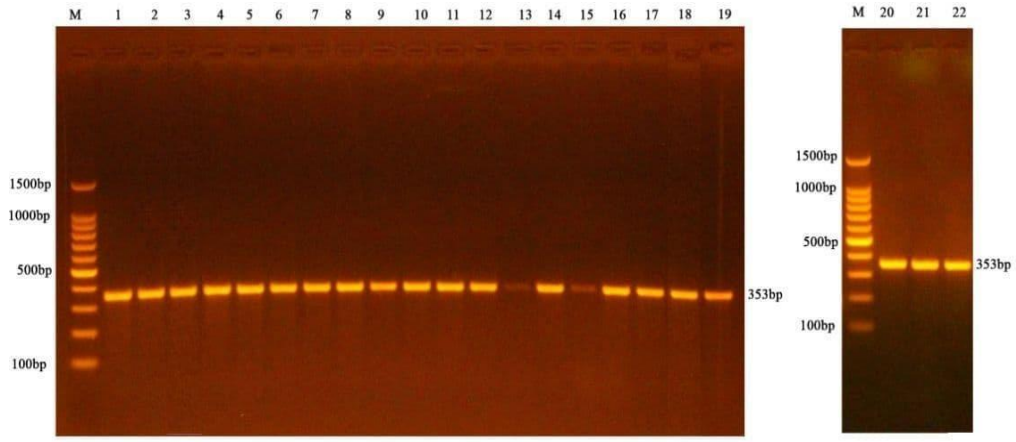

FIGURE 2 : The amplification results usingblaoxa51 primer in *Acinetobacterbaumannii* speciesfractionatedon1.5%agarosestainedwithEth.Br. M:100bpladder marker.Lanes1-22resemble353bp

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PCRproducts.

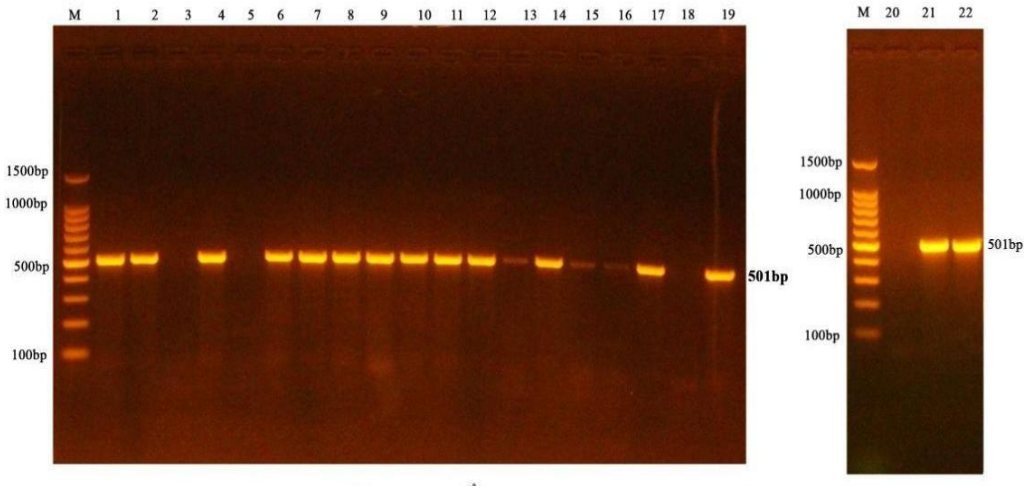

FIGURE 3 : The amplification results using blaOXA-23primer in *Acinetobacterbaumannii*fractionatedon1.5%agarosestained withEth.Br. M:100bpladder marker.AllLanes werepositive 501bp PCRproducts,expect3,5,18 also20werenotcarrying blaOXA-23.

DISCUSSION

From September (2020) to March (2021) 22 A.baumanniiisolates were obtained from clinicalsamples of (burns, wounds, urine, blood and fluids). Biochemical measures such as sugar fermentation (+),citrate utilization (+), urease(-),motility (-), oxidative/fermentative glucose (O/F) test(-),oxidase (-),catalase(+), and growth potential at 37°C and 42°C (+) were used to identify*A. baumannii*isolates(10).Theisolateswereclassifiedatthegenuslevelusingthe VITEK 2 automated method (biomérieux , France). however, hasbeenshowntobeineffectiveindistinguishing*Acinetobacterbaumannii*fromotherbacteriasuchas*Acinetobacternosocomialis*,*Acinetobacterpitii*,and*Acinetobactercalcoaceticus* (14). Presence of bla_{OXA-51} in all isolates was also investigated .(FIGURE 1).

Antibioticsusceptibility testing:

22 carbapenem resistant A. baumannii were tested to show antibiotic susceptibility were (71.5 %) (68.75%) . Infections caused by Acinetobacterspp treated by carbapenem .This finding with close research published by (Hameed A. M. and Najeeb L.M.,2020) (15), that (68.75%) carbapenemresistant A. from 48 clinical strains were multidrugresistant.

Detectionofbla_{OXA-23}andbla_{OXA-51}inA. buammaniiby molecularassay :

MDRA.*baumannii*clinicalisolateshaveahighprevalenceofblaOXA-2318accordingtoPCRfindings(81 %). *A. baumannii* has been shown to release OXA-23 in a number of republics, suggesting that thisenzyme is commonly spread on the globe (16). Nosocomial outbreaks or sporadic cases of *Acinetobacter* containing OXA-23 were published in(Europe, Singapore, Australia, the United States, Algeria,Egypt, Libya, South Africa, Thailand, Tunisia, Iraq, and French Polynesia) this research by Kempf and Rolain(17). In *A. baumannii*, OXA-23 is the most commonly distributed class D enzyme in China and Korea (18). OXA-23-like enzymes havebeenidentified asoneof thekeycarbapenemresistancepathways in*Acinetobacter*spp. in Brazil.(19,20).

The blaOXA- 51 gene has been described as being particularly specific for distinguishing *A. baumannii* atthe

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species level (21). The expression of blaOXA-51 genes was detected in all 22 isolates (100%) of *A. baumannii*by PCR, which is compatible with previous studies in Iraq. Mohammed S.M. (2020)(22) discoversthe same thing in the city of Diyala. Another research in Anbar/IRAQ discovered bla_{OXA-51} gene inall CRAB isolates, suggesting that the bla_{OXA-51} gene is the most common mechanism for imipenemtolerance in *A. baumannii* isolates. (Hameedand Najeeb) (15). Anane et al.,(2020)observed that bla_{OXA-51} was present in all isolates, while bla_{OXA-23} was present at 70% ofisolates (22 ,23).

Nonetheless, in countries other than Iraq, such as the United States, similar findings have been released (Bulgaria,China,Brazil, Afghanistan also Korea .(21) Both isolates contained the chromosomally encoded bla_{OXA-51} gene, corroborating previousobservations and suggesting that the discovery of the bla_{OXA-51} gene may be used as a supplementarytoolfor recognizing*A.baumannii*atthe species levelincombination withother procedures(24).

CONCLUSION

we detectahigh occurrence of bla_{OXA-51} and bla_{OXA-23} in multidrug resistance *A. baumannii* strainsin Baquba Teaching Hospital, Diyala , Iraq, . Due to the difficulty of selecting empirical medications for chronically sick people, there is a risk of additional hospitalization and related costs. Continuous research, as well as early identification of MDR *A. baumannii* isolates, is critical in order to prevent their dissemination inside the hospital setting.

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