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OCCURRENCE OF GENETIC DETERMINANTS OF RESISTANCE TO COLISTIN AND BIOFILM ABILITY IN CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII* ISOLATED FROM HOSPITALIZED PATIENTS

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INTRODUCTION & AIM OF THE STUDY

Non-fermenting, Gram-negative, carbapenem-resistant *Acinetobacter baumannii* (**CRAb**) has been identified by the World Health Organization (WHO) as a priority pathogen, representing the most significant threat to human health. CRAb is responsible for a range of infections, including pneumonia, bacteraemia, urinary tract infections, and skin and soft tissue infections. Mortality rates associated with these infections are approaching 35%, which is a significant concern in the field of medicine [1].

The aim of the study was to evaluate the occurrence of plasmid-mediated colistin resistance (*mcr1-5*) and biofilm-related genes (*surA1*, *bap*, *ompA*, *luxR*, *epsA*) in a total of 26 CRAb isolates collected from hospitalised patients and *Acinetobacter baumanii* ATCC 19606 as the reference strain.

METHODOLOGY

Twenty-six bacterial isolates of *A. baumannii*, carbapenem-resistant (previously assessed by disk-diffusion method), from the collection of the Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland, were included in the study. All microbes were collected from intensive-care unit patients from various specimens (urine, blood, etc.). Isolated were identified by the VITEK Compact 2 system (bioMerieux, France) and the growth on the Chromagar *Acinetobacter* LAB-AGAR™ (Biomaxima, Poland; **Figure 1**).

Figure 1: Images of the (**A**) gram-staining (magnification of 1000x, Olympus light microscope; scale 5 µm) and (**B**) growth on the Chromagar *Acinetobacter* LAB-AGAR™ (Biomaxima, Poland).

The genes encoding plasmid-derived resistance to colistin and the prevalence of biofilm-related genes were determined by multiplex or single PCR reactions, respectively, in a total volume of 25 µL mixture containing 12.5 µl of REDTaq® ReadyMix™ PCR Reaction Mix (Merck, USA), 1.0 µl of each primer (Genomed, Poland; the sequences are provided in **Table 1**), 8.5 µl of free-nuclease water (EURx, Poland) and 2.0 µl of bacterial genomic DNA template. The reaction temperature-time conditions for the *mcr1-5* genes were according to [2]. Other reactions aplifying biofilm-related genes were performed according to [3,4].

All determined amplicons were evaluated using electrophoretic separation in a 1.5% agarose gel with SimplySafe dye (EURx, Poland) with Perfect™ 100-3000 bp DNA Ladder size marker (EURx, Poland). Electrophoresis was carried out at a constant voltage of 120 V/cm for 35 minutes. The results were analysed and archived using the Quantum ST5 Xpress v 16.08g electrophoretic image analysis and documentation software (Vilber Lourmat, France).

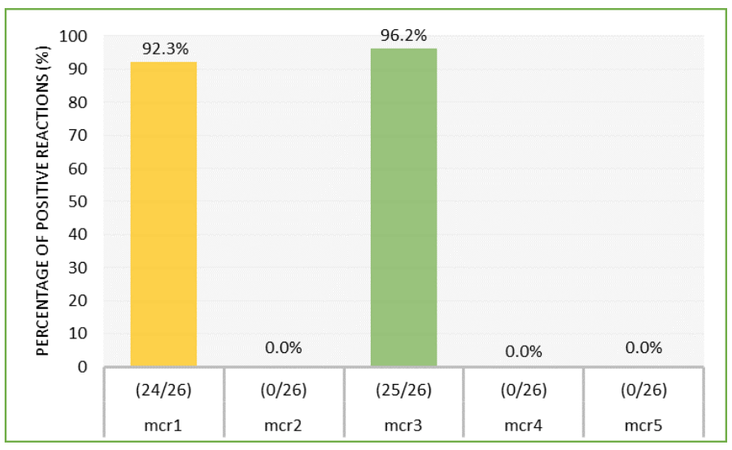
Table 1: Primer sequences of colistin drug resistance genes and biofilm-related genes, as identified through PCR reactions, among carbapenem-resistant *Acinetobacter baumannii*.

Name of detected genes		Primer sequence [5′ → 3′]	Product size [bp]	Ref.
plasmid-determined colistin resistance	*mcr1*	AGTCCGTTTGTTCTTGTGGC	320	[2]
		AGATCCTTGGTCTCGGCTTG		
	mcr2	CAAGTGTGTTGGTCGCAGTT	715	
		TCTAGCCCGACAAGCATACC		
	mcr3	AAATAAAAATTGTTCCGCTTATG	929	
		AATGGAGATCCCCGTTTTT		
	mcr4	TCACTTTCATCACTGCGTTG	1100	
		TTGGTCCATGACTACCAATG		
	mcr5	ATGCGGTTGTCTGCATTTATC	1644	
		TCATTGTGGTTGTCCTTTTCTG		
biofilm-related	*surA1*	CAATTGGTAGCTGGCGATCA	241	[3]
		TTAGGCGGGACTCAGCTTTT		
	bap	GAGGGAACTTCTGCAAAACTTTC	108	[4]
		CAGACGTATGACTGCATTGGT		
	ompA	GAGTCGTATTGCACTTGCTAC	594	
		GCAGGCTTCAAGTGACCACC		
	luxR	AGCCCTAGCATTACAGCTCG	631	
		CTACCGCATCAAGGCTCGGAT		
	epsA	AAACATTACCAGCGATACAACC	602	
		CTGGTTTTCTCGTGTGCTGAC		

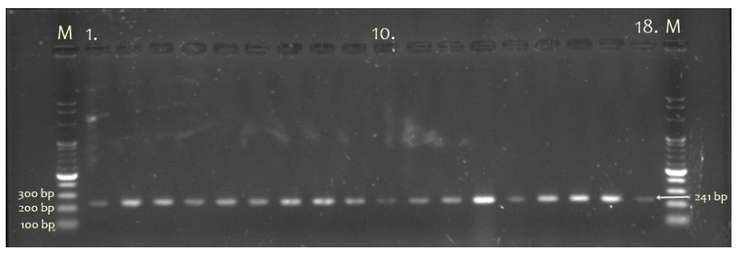
RESULTS & DISCUSSION

The prevalence of five plasmid-mediated colistin resistance genes was determined through multiplex-PCR reactions (**Figure 2**). Of the *A. baumannii* isolates, 92.3% (24/26) and 96.2% (25/26) were found to be *mcr1*- and *mcr3*-positive, respectively. No isolates exhibited the presence of *mcr2*, *mcr4* and *mcr5* genes. Only 23.1% (6/26) of isolates had a single the *mcr3* gene with simultaneous absence of the *mcr1* gene. These results are in line with those of Sobieh et al. among pandrug resistant *A. baumannii* [5], but not with Lowe et al. [6] in South Africa or Hameed et al. [7] in Pakistan.

Figure 2: Results of multiplex-PCR reaction for plasmid encoded *mcr1-5* genes among carbapenem-resistant *Acinetobacter baumannii*.



All CRAb isolates, collected from hospitilized patients were characterized by diverse prevalence of biofilm-related genes, with the *surA1* (**Figure 3**), *bap*, *ompA* and *luxR* (26/26; 100.0%) as the most common, followed by 61.5% (16/26) of isolates presented the *epsA* gene product (**Table 2**). These results are in agreement with Depka et al. in Poland [3] and Al-Shamiri et al. in China [4], especially for *Acinetobacter* spp. strong biofilm producers. The *surA1* and *bap* genes are considered by these authors as genetic markers for biofilm formation.



Abbreviations: **M** – 100-3000 bp marker size; lines **1-18** – *Acinetobacter baumannii* isolates

Figure 3: Electrophoretic gel image after amplifying of the *surA1* gene in the *Acinetobacter* spp. isolates tested.

Table 2: Results of multiplex PCR reaction for plasmid encoded biofilm genes among carbapenem-resistant *Acinetobacter baumannii*.

Gene	Number of isolates with detected gene	Positive isolates [%]
surA1	26/26	100.0
bap	26/26	100.0
ompA	26/26	100.0
luxR	26/26	100.0
epsA	16/26	61.5

Table 3: Genotype distribution among carbapenem-resistant *Acinetobacter baumannii* isolates.

	Genotype	No. of isolates	[%] of isolates
A	*mcr1-mcr3-surA1-bap-ompA-luxR-epsA*	16	61.5%
B	*mcr1-mcr3-surA1-bap-ompA-luxR*	13	50.0%
C	*mcr3-surA1-bap-ompA-luxR*	3	11.5%
D	*mcr3-surA1-bap-ompA-luxR-epsA*	3	11.5%
E	*mcr1-surA1-bap-ompA-luxR-epsA*	1	3.8%

The results indicated that all CRAb isolates exhibited five distinct genotypes (**Table 3**), with the genotype A *„mcr1-mcr3-surA1-bap-ompA-luxR-epsA"* being the most prevalent (61.5%, 16/26), presenting the presence of all genes tested, followed by the genotype B *„mcr1-mcr3-surA1-bap-ompA-luxR"*, which was identified in 50.0% (13/26) of isolates.

CONCLUSIONS

Over 90% of the CRAb isolates were found to carry the *mcr1* and/or *mcr3* plasmid-mediated colistin resistance genes. At the same time, all *Acinetobacter* spp. isolates had at least four biofilm-related genes, including the *surA1, bap, ompA* and *luxR*. 61.5% of them also had the *epsA* gene. The genotype A *„mcr1-mcr3-surA1-bap-ompA-luxR-epsA"* was found to be the most common in 61.5% of the isolates. The results suggest that a much larger number of genes may be involved in the biofilm formation process. To better understand it, studies need to be expanded to include more species. Given the high pathogenicity of these bacteria and the lack of data on them, it is important to include them in the future research objectives. Every effort should be made to prevent the spread of these microorganisms, including analysing the presence of new genetic determinants in the CRAb genome, and identifying biofilm-related genes whose expression inhibition could more effectively eradicate these pathogens.

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