DETECTION OF 11 MULTIDRUG RESISTANCE GENES AMONG THE STRAINS OF *A.BAUMANNII* BY COMPUTATIONAL APPROACH

ABSTRACT:

BACKGROUND: *Acinetobacter baumannii* is typically short, rod shaped gram negative bacterium. The World Health organisation has declared it as an opportunistic pathogen in humans. Multi drug resistance involves different genetic determinants making the pathogen difficult to treat. So this study is undertaken to characterise the 11 different drug resistant genes from 19 virulent strains of *A.baumannii* using in-silico PCR.

AIM: To detect the 11 multidrug resistance genes among the strains of *A.baumannii* by computational approach.

MATERIALS & METHODS: 11 multidrug resistance genes of *A.baumannii* were selected. Forward and reverse primers of the 11 genes as reported from earlier studies were used for in-silico PCR amplification. 19 strains of *A.baumannii* set as default on the server were chosen and the amplicon bands were observed.

RESULT: Among the 11 multidrug resistance genes only blaOXA-51like and blaADC were detected among the 19 virulent strains of *A.bauamannii*.

CONCLUSION: The findings of the study documents the frequency of blaADC and blaOXA-51 like from the selected strains of *A.baumannii*. However further experimental validation must be done towards the periodical surveillance on the drug resistant strains of *A.baumannii* in hospital settings.

Key words: *A.baumannii*; novel blaOXA; adc; blaOXA; resistance: environmental strains; innovative in-silico

Running title: MDR genes among the strains of *A.baumannii*

INTRODUCTION:

Acinetobacter baumannii is typically short, almost round, rod shaped gram negative bacterium. WHO (World Health Organisation) has declared it as an opportunistic pathogen in humans, affecting people with compromised immune systems and is becoming increasingly important as a hospital derived infection (1). *A.baumannii* are multidrug resistant bacteria, which have characteristics such as aerobic, pleomorphic, non-motile and coccobacilli bacteria. *A.baumannii* is classified under the moraxellaceae family, which remains difficult to treat. The prevalence of drug resistant strains are continuously increasing and therefore the treatment options are considerably limited (2). Propensity of multi-drug resistance in *A.baumannii* is a significant reason for its major transformation as a nosocomial pathogen. There are three different types of multidrug resistance properties, each has their own systemic quality. MDR-*A.baumannii*, alludes to strains which display protection from more than at least three antimicrobial drug classes. XDR-A.baumannii, alludes to all strains impervious to everything except two medication classes. Pan-drug resistance, alludes to opposition shown by the strains to all medication classes, and a development of culture safe *A.baumannii* was accounted for including the segregates that were impervious to carbapenems, colistin and polymyxins (3). This is due to multiple mechanisms of drug resistance shown by *A.baumannii* via impermeable outer membrane, production of enzymes such as different beta lactamases classes wich allows resistance towards carbapenems, porin channel alteration, efflux pumps and genetic materials that leads resistance towards fluoroquinolones (4).

Carbapenems have an ability of hydrolysing the beta lactamases (*carbapenamases*) which belong to molecular class D (OXA enzyme) that have constantly emerged around the globe with high prevalence in East Asia. The class D (OXA carbapenems) of *Acinetobacter* species are divided into several phylogenetic subgroups: blaOXA-23like, blaOXA-51like, blaOXA-141like. In recent findings it has been reported that enzymes belonging to the subgroups blaOXA-51 like are

intrinsic to *A.baumannii*, that occurs in most (or) all strains, even though very variably expressed (5). There were identification of two clones showing carbapenems resistance, these are produced by blaOXA-23like enzymes, they are named as OXA23 clone-1 and OXA23 clone-2.The third group called as SE clone, shows variable carbapenem resistance acquired by insertion sequence (6).

According to Ambler classification, the taken enzyme belongs to class-B metallo-beta lactamase (MBLs) and the multidrug resistance class D OXA type carbapenemases and most of them are mediated by plasmids. MBL's are further divided into several groups such as blaVIM, blaIMP, blaGIM and a recent finding has documented blaNDM in almost many clinical strains. In the MBL's activity the presence of divalent cations are needed as co-factors with the action of one or two zinc ions, for the catalytic ability of chelating as an inhibitory agent. All these mentioned 4 MBL's are having high potential for hydrolysing all beta-lactam antibiotics except for the specific monobactams such as aztreonam (7), (8).

Most studies have documented *A.baumannii* as MDR strains, and show high variations in both their phenotypes and genotypes as nosocomial pathogens. blaTEM, blaSHV-type and blaCTX-type of genes, are responsible for extended spectrum beta-lactamase production that could be mediated by both plasmids and chromosomes. This plays an important role in exhibiting the resistance against later generations cephalosporins such as cefepime, cefotaxime and ceftazidime (9).

In the same line, sulbactam is a beta lactamase inhibitor, when combined with penicillin it lacks the ability of antimicrobial activity in most bacterial species. It possesses both bacteriostatic and bactericidal effects against *A.baumannii* through several mechanisms. With many studies documenting the drug resistance properties among *A.baumannii*, the present study was undertaken to evaluate the frequency of 11 genetic determinants of resistance genes among the 19 different strains of *A.baumannii* by computational approach.

MATERIALS & METHODS:

Study Setting:

This is an observational in-silico study done in the Department of Microbiology, Saveetha Dental College and Hospital. Institutional approval for the research was obtained (IHEC/SDC/UG-1895/21/151).

This is an original research study where we have selected 19 strains of *A.baumannii* set as default in the in-silico PCR server (10). The genes of target were blaOXA-23like, blaOXA-51like, blaOXA-141like, blaVIM, blaIMP, blaGIM, blaNDM-1, blaTEM, blaSHV, blaCTX and blaADC. Upon the amplification command, the server produced the amplicon bands for evaluation of the band size. From the amplicon bands, the frequency of the distribution of the drug resistant genes among the vital virulent strains of *A.baumannii* were evaluated and compared. Further evolutionary relationships were compared with the phylogenetic analysis as done in earlier reports (11) (12).

RESULTS:

The investigation on the prevalence of the drug resistant genes from 19 different strains of *A.baumannii* (Table 1) using an in-silico amplification server was promising. The results showed the starting position of the amplification in the chromosome or plasmid and the length of each amplicon. Amplicons obtained in each chromosome or plasmid have been tabulated (Table 2) with target genes, primers used, sequenced of primer (5' to 3'), annealing temperature, estimated size of base pair and the frequency of the target genes among the study strains. Among the 11 multidrug resistant genes we observed 78.94% positivity of blaOXA-51like 68.42% for blaADC. All the other 9 genes were not present in the selected 19 different strains of *A.baumannii*. We further assessed the evolutionary pattern of the distributed genes among the strains.

Table 1 showing the 19 strains of *A.baumannii* selected for the study

S.No	***A.baumannii strains under study***
1	Acinetobacter baumannii 1656-2 chromosome
2	Acinetobacter baumannii AB0057
3	Acinetobacter baumannii AB307-0294

4	Acinetobacter baumannii ACICU
5	Acinetobacter baumannii ATCC 17978
6	Acinetobacter baumannii AYE
7	Acinetobacter baumannii BJAB07104
8	Acinetobacter baumannii BJAB0715
9	Acinetobacter baumannii BJAB0868
10	Acinetobacter baumannii D1279779
11	Acinetobacter baumannii MDR-TJ
12	Acinetobacter baumannii MDR-ZJ06
13	Acinetobacter baumannii SDF
14	Acinetobacter baumannii TCDC-AB0715
15	Acinetobacter baumannii TYTH-1
16	Acinetobacter baumannii ZW85-1
17	Acinetobacter calcoaceticus PHEA-2
18	Acinetobacter sp. ADP1
19	Acinetobacter sp. DR1

Table 2 showing the target genes, PCR conditions and the prevalence of the genes among the selected strains of *A.baumannii*

Target	Primer	Sequence 5' to3'	AT (°C)	ES (bp)	Bands	Virulence %
blaOXA-23-like	OXA-23-F	GATCGGATTGGAGAACCAGA	52	501	No bands	Nil
	OXA-23-R	GATCGGATTGGAGAACCAGA				
blaOXA-51-like	OXA-51-F	TAATGCTTTGATCGGCCTTG	52	353	15	78.94%
	OXA-51-R	TGGATTGCACTTCATCTTGG				
blaOXA-143-like	OXA-143-F	TGGCACTTTCAGCAGTTCCT	52	149	No bands	Nil
	OXA-143-R	TAATCTTGAGGGGGCCAACC				
blaVIM	VIMgen-F2	GTTTGGTCGCATATCGCAAC	53	382	No bands	Nil
	VIMgen-R2	AATGCGCAGCACCAGGATAG				
blaIMP	IMPgen-F1	GAATAGAATGGTTAACTCTC	53	188	No bands	Nil
	IMPgen-R1	CCAAACCACTAGGTTATC				
blaGIM	GIM-F1	TCAATTAGCTCTTGGGCTGAC	53	72	No bands	Nil
	GIM-R1	CGGAACGACCATTTGAATGG				
blaNDM-1	NDM-Fm	GGTTTGGCGATCTGGTTTTC	52	621	No bands	Nil
	NDM-Rm	CGGAATGGCTCATCACGATC				
blaTEM	TEM up	ATGATGATTCAACATTTCCG	52	858	No bands	Nil
	TEM low	CCAATGCTTAATCAGTGAGG				

blaSHV	SHV up	TTATCTCCCTGTTAGCCACC	50	795	No bands	Nil
	SHV low	GATTTGCTGATTTCGCTCGG				
blaCTX-M	CTX-MA	CGCTTTGCGATGTGCAG	55	550	No bands	Nil
	CTX-MB	ACCGCGATATCGTTGGT				
blaADC	ADC1	CCGCGACAGCAGGTGGATA	51	420	13	68.42%
	ADC2	TCGGCTGATTTTCTTGGTT				
	125R	TAGACGTAGACGTGGTCA				

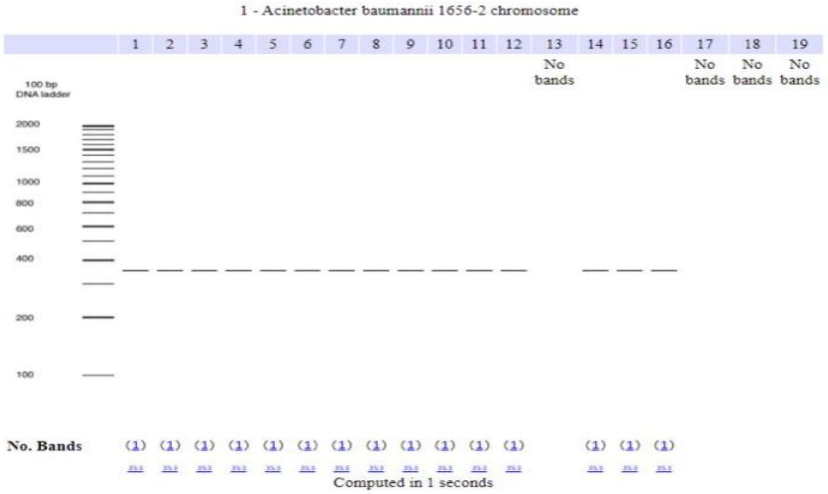

Figure 1 showing the positive band formation for blaOXA-51like using in-silico PCR amplification among the strains of *A.baumannii*

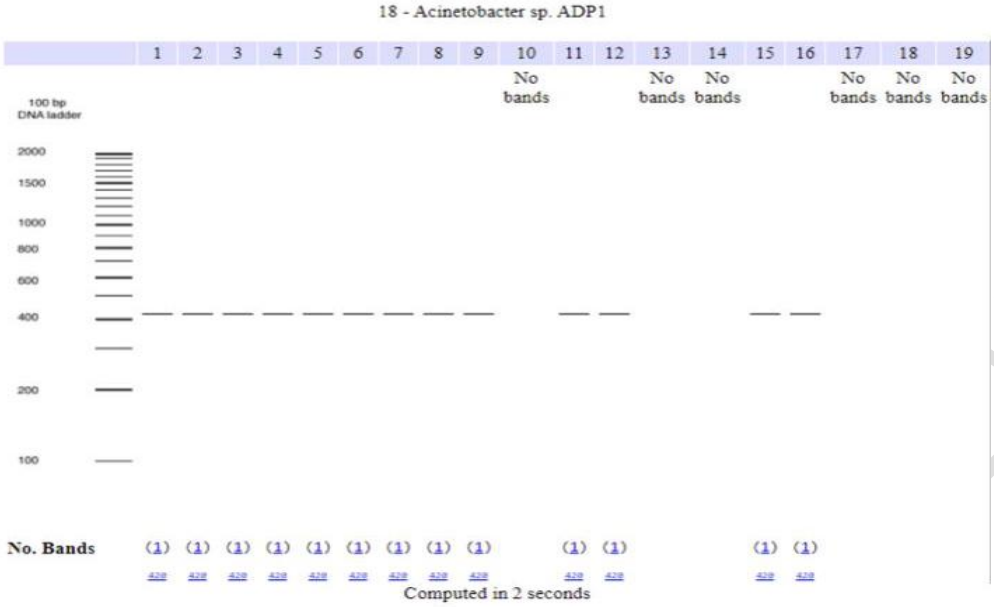

Figure 2 showing the positive band formation for blaADC using in-silico PCR Amplification among the strains of *A.baumannii*

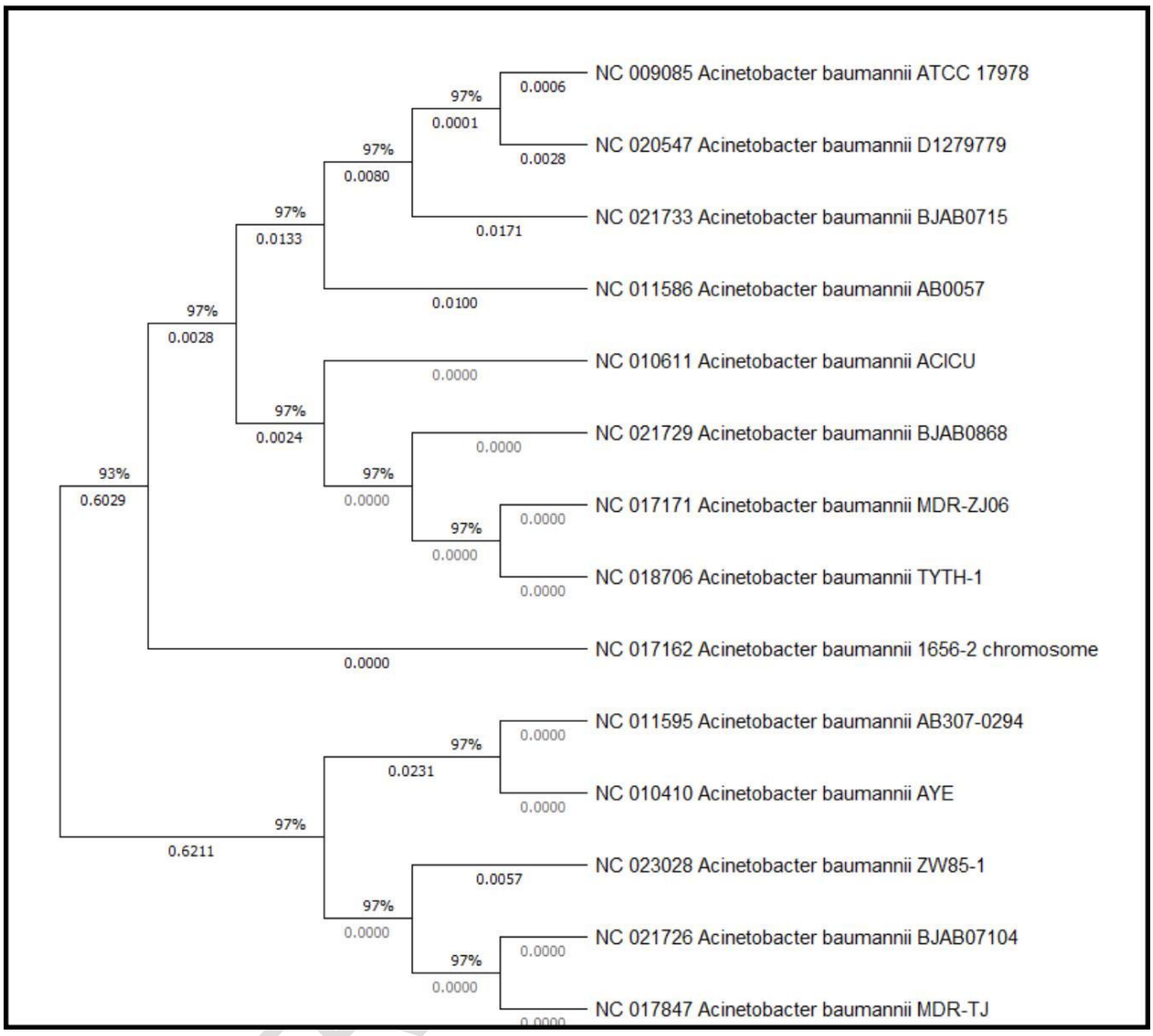

Figure 3 showing the positive phylogenetic tree construction for blaOXA-51like using in-silico PCR Amplification among the strains of *A.baumannii*

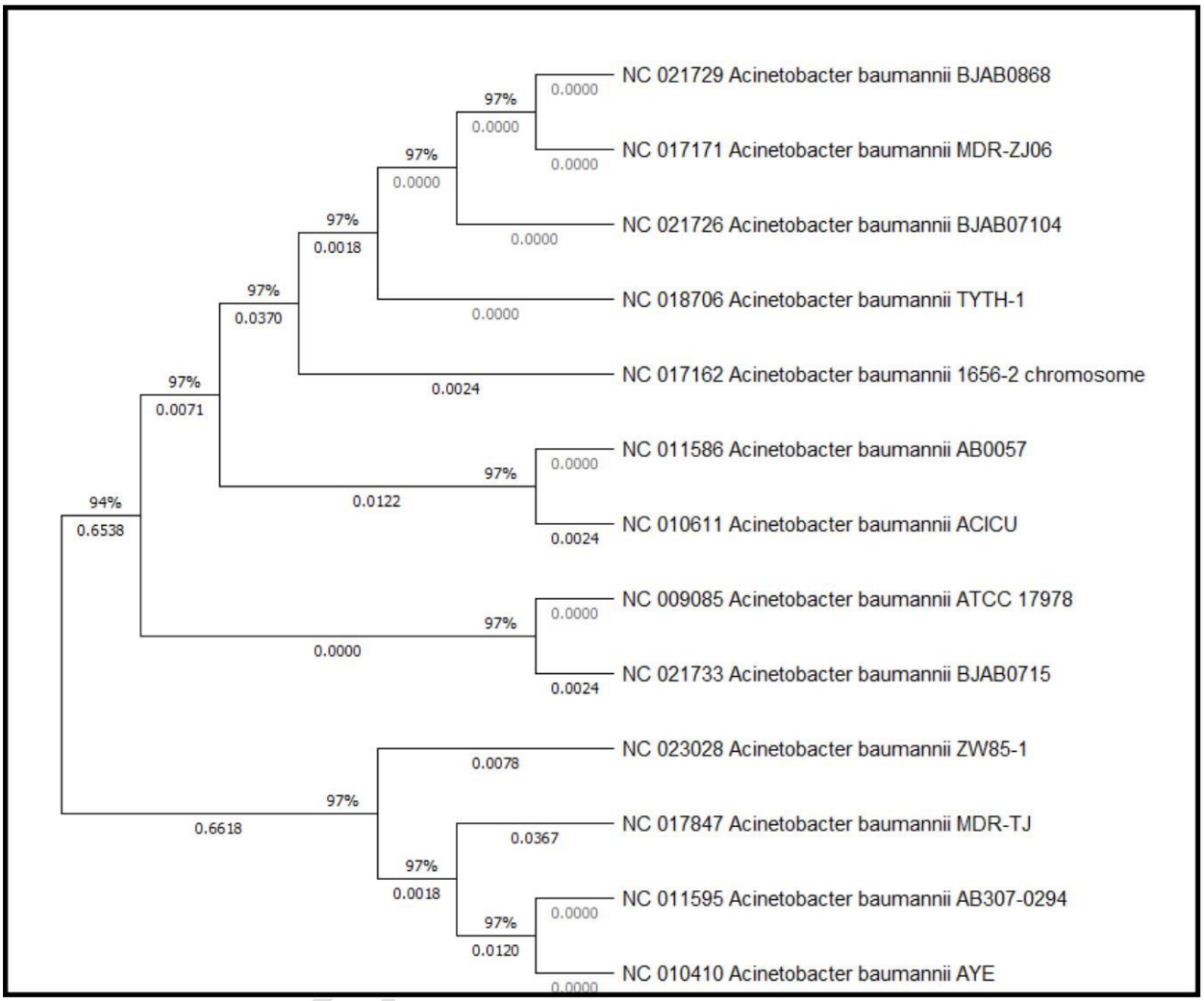

Figure 4 showing the positive phylogenetic tree construction for blaADC using in-silico PCR Amplification among the strains of *A.baumannii*

DISCUSSION:

Higher frequency of blaOXA51 and blaADC, were observed in the present study. In the study we have found the virulence of the blaOXA-51like and blaADC as 78.94% [Figure 1] and 68.42% [Figure 2] respectively there was a similar study representing the higher virulence factor than our study. In the previous study it has shown that 93% of virulence factors are seen for blaOXA-51like (13). There was a study reporting that the found blaADC have higher virulence when compared to our current study (14). Our team has extensive knowledge and research experience that has translated into high quality publications (15–23)[Table-1].

There were studies done in finding the targeting NM23-H1-mediated inhibition of tumour metastasis, targeting EBNA-1, virulence factors of nano-zinc oxide varnish, enamel defects in infants and parental SARS-CoV-2 fear and distress based on coronavirus disease (24–28).

Also there have been other studies stating that there were no higher frequencies of blaOXA-51like [Figure 3] and blaADC [Figure 4] also they have reported that there was only a low frequency of the genetic determinants. In an earlier study (29) they have reported a higher frequency for metallo-beta lactamases enzymes and no detection of Class D (OXA enzymes) on the strains of *A.baumannii*. In the earlier studies (30) detection of higher frequency of extended spectrum beta lactamase producing strains were observed with low frequency of other resistant genes. In Silico identification of genes like csgA among ESBL strains, and can also be applied for various other systemic and immunological related disorders as well (31–35).

Thus the present findings of the study were in both correlating and in contrast with many other earlier studies. This suggests, the in-silico PCR amplification is best suited for the preliminary identification of the vital genes. However, further studies with the clinical strains in-vitro can render the actual results on the prevalence and distribution of the virulent and resistant genes among *A.baumannii*. The limitation of this study is that it involved only the set default strains in the tool, thus requiring the same evaluation with the clinical strains. The limitation of the study was that the distribution of the resistant determinants was observed as a computational approach. Thus the future prospects are set to evaluate the same using specific in-vitro and in-vivo study models.

CONCLUSION:

The present study had detected the frequency of two vital genetic determinants of resistance among the 11 genes targeted for the study. blaOXA-51 like and blaADC were highly distributed among the selected 19 strains *A.baumannii*. The *in-silico* PCR tool was efficient in the identification of the genetic determinants at a preliminary level. However, further experimental evaluations must be done using the clinical isolates and to periodically monitor the resistance pattern for epidemiological surveillance of *A.baumannii* associated infections.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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