IOSR Journal of Nursing and Health Science (IOSR-JNHS)
e-ISSN: 2320–1959.p- ISSN: 2320–1940 Volume 9, Issue 3 Ser. VI (May - June 2020), PP 36-42
www.iosrjournals.org

"Antagonistic Effect of Homoeopathic Preparations on Acinetobacter baumannii, A Common Nosocomial*Infectious* Agent through In Vitro Study"

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Abstract
Background:*Acinetobacter baumanniiis a common cause of nosocomial infection in hospital & community. It can survive in any environmental condition.World Health Organization(W.H.O)already declared it's a severe problem in health sector & they categorised it in a priority 1(critical) list due to its emerging ability ofMulti Drug Resistance (MDR).Homeopathy may have a role to play in combating the development ofantibiotic resistance.*
Materials and Methods: *Clinically Isolated Samples were collected from BharatiVidyapeeth hospital, Pune& incubated at 37°C to growth of A. baumannii. Sample had tested for MDR category under Kirby-Bauer process.An in-vitro anti-microbial Studies were performed of various Homoeopathic remedies with their different dilution&Tested against clinically isolated pathogenA.baumannii. We were compared with Vehicle control (90% ethanol+media+culture), Negative control (Media+Culture), Media control (Media+Solvent), Mother Tincture & Meropenem is a Positive control.*
Results:*Homoeopathic drug Bryonia, Lachesis&Belladonna different potencies were tested against pathogenic A. baumannii.6CH potency of Bryonia&Lachesisshows good results* ***(0.115 ±0.005,0.130 ± 0.0140)*** *as compared to other potencies. In Kirby-Bauer test we found that all pathogenic clinically isolated samples were ability to MDR category &its measurement of zone of inhibition confirmed by the Clinical and Laboratory Standards Institute(CLSI)guideline& Maximum sample was resistance (≤11/≤12) to the multiple antibiotics like, Ampicillin, Amoxiclav, Cefotaxime, Co-Trimoxazole, Gentamicin, Tobramycin etc. Except Meropenem.Hence it was selected as a positive control. Results of disk diffusion expels as compared to modern medicine confirmed 6CH potency of Bryonia& Lachesis as an effective measures against A.baumannii****(p<0.005).****The results indicate inhibited potencies of homoeopathic medicine against A.baumanniithat would be helping in prevents nosocomial spread.*
Conclusion: *-This result of that experiments supports that concept of "evidence-based medicine" in Homoeopathy. In future Homoeopathic medicine should be used in nosocomial infection cases. The claim can be substantiated further by In-vivo experimentation.*
KEYWORDS*: -Acinetobacterbaumannii, WHO,CLSI,Anti-bacterial activity, 96 Broth dilution method, Bryonia, Belladona, Lachesis, Kirby-Bauer test, AntibioticResistance, Homoeopathy& Antibacterial Activity.*

Date of Submission: 22-05-2020 Date of Acceptance: 09-06-2020

I. Introduction

Infectious diseases are very common in developing countries due to environmental changes, pollutedsoil, water& also lower, lower -middle class economic country.Antibiotic-Resistant/multi drug resistance plays a major role to produce infection [4,5,9]. Mainly those who are seriously ill, Hospitalised patients, immunocompromised host, Chronic lung diseases, Diabetic patients, openwound, prolongedhospital stay or long-term care setting, Invasive medical devices& prolonged antibiotics use are some of the major reasons for the spread of nosocomial infections & MDR. As a general timeline infection occurring more than 48 hours after admission are usually considered hospital acquired [5,8,9].

Recant study report show that gram-negative bacterialike, *Acinetobacter baumannii,Escherichia coli, Klebsiella pneumonia* etc. are the main cause of death in the Hospital & community acquired infections specially I.C.U patient due to its MDR ability [4,5,9]. As per W.H.O criteria they are categorised in 3 point like,

Critical, High & Medium. *Acinetobacter baumannii* is the 1st present/ mention on this Priority-1(critical)category. [1,11,15]

Acinetobacter baumannii is a group of aerobic, glucose-non- fermentative, non-motile, non-fastidious, catalase-positive, oxidative- negative, gram-negative coccobacillus [10]. In gram staining pairs ranging from 1to1.5 mm. It often resists complete decolorization and can deceive as gram-positive cocci. Colonisers 1.5-2.0 mm in diameter after 24 h & 3.0-4.0 mm in diameter after 48 hours at15°C-44°C [10]. In hospital environment they can grow on beds, curtains, wall, roofs, medicaldevices, equipment, Handsanitizers, dispensary's etc. not only that, it can grow in different environment. They have the capacity to survive for prolonged periods on inanimate objects.[2] *Acinetobacterbaumannii* is an important cause of nosocomial infection/ Hospital acquired infection. It is producedbacteraemia, pneumonia, ventilator -associated pneumonia, meningitis, urinary tract infection, catheter- associated UTI, central line- associated bloodstream infection, Wound infection [5,7,8,9].

Homoeopathic is the 2nd largest worldwide accepted alternative system. It has no side effect in human body like antibiotics.Policies to restrict use of antibiotics have limited success. Homeopathy may have a role to play in combating the development ofantibiotic resistance**.**Homoeopathic preparation *like Bryonia, Belladona& Lachesis* are covered all indication of *Acinetobacter baumannii*producedsymptoms. The objectives of the present studyis to determine the best potency showing max. effect on *A.baumannii*& further to confirm the efficacy by in-vitro determination fulfilment against MDR*A.baumannii.*

II. Materials &Methods

Media &chemicals: -All media & chemical were procured from HI-Media Lab, Mumbai. Meropenem antibiotics was purchased from Local medicine shop, Dispensing Alcohol (ethanol 90%) according to Homoeopathic pharmacopeia of India Vol-1.

Sample Culture: -AllclinicallyIsolated culture of *Acinetobacterbaumannii*were collected from Bharati Vidyapeeth Medical college (Microbiology lab). Protocol was approved by institutional ethical committee consent was obtained from the hospital administration & academic committee of the hospital for use of anonymized data of the patient, Individual patient consent was not obtained.

Homoeopathic Medicine: - All Homoeopathic preparation were collected from the Swabe India Pvt.Ltd**.** ***Bryonia* 6Ch to MT, *Belladona*6CH to MT, *Lachesis* 6CH to 10M** [12]

Organism: -Total isolates cultures were obtained from Bharati VidyapeethHospital, Pune. The Cultures were Cultivated Luria Agar at 37°C by Overnight incubation.
The organism to be tested should be subculture using a Luria Broth medium under optimal incubation condition (37°c) to obtained a fresh overnight grown culture (Test tube media is 100 ml L.B)
After overnight incubation, the streak culture was checked for purity & turbidity tested by Spectrophotometer.
Preparation & inoculation of the dilution series: -
The optical density of the overnight culture of the strain is determined Spectrophotometrically at 600 nm & it is standardized at 1±0.02(i.e. app.10E8 CFU/ml) by dilution with sterile ISB.

96 Wells Broth Dilution method: - Initially experiment was performed in 96 well microtiter plate. Each well contained 130µl media + 20µl culture (overnight)& 100 µL sample. Following controls were used: - Vehicle control (90% dispensing alcohol in 100 µl +130µl media/Broth+2oµl culture),
Negative Control taken 230µl broth+ 20µl culture, Media control: - Media/Broth 150µl+100µl solvent used respectively& Positive control (130µl Broth+100µl meropenem+20µl culture).
Following Δ^n at 37°c O.D was measured at 600nm.
Antibiotic sensitivity/Kirby-Test: -

This test was done in an Agar well diffusion or Kirby-BauerprocessBacteria were classified as susceptible, intermediate or resistant to antibiotics in accordance with current Clinical Laboratory Standard Institute (CLSI) recommendation. [3,6] For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, centre to centre. Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Holding the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the aided eye. Ignore faint growth of tiny colonies that medium may allow some slight growth; Therefore, Disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margined to determine the zone diameter [16]. So, in this circumstance we were clearly understood that the isolated sample was an MDR character.

Agar Well Diffusion Method/Ditch (punch well) Method: -

Punch wells of 6mm diameter are made with the aid of sterile metallic template, on the surface of agar plates. Those potencies giving the best results in microtiter plate assay were selected for this experiment. Plates are incubated with organisms and various potencies of *BELLADONNA, BRYONIA ALBA, LACHESIS MUTUS 6CH & 200 CH*will be delivered off into each of the wells along with *Meropenem*. After incubation, Zone inhibition are observed and evaluated.

Statistical Analysis: All experimental study was performed in a Duplicate/Triplicate value. Mean &Standered Deviation values was Calculated by the help of Microsoft Excel & GraphPad Prism 8.0.3. The One-way ANOVA test was used for calculate the statistical significance of p-value ($p<0.005$). The percent of inhibition was calculated by the compare count of Vehicle Control as 100%.

III. Results

Clinically isolates of A.baumannii bacterial sample wereobtained from Bharati Vidyapeeth Medical Hospital,Pune.

Kirby-Bauer process /Antibiotic sensitivity Test: -

This test was done in an Agar well diffusion or Kirby-Bauer process Bacteria were classified as susceptible, intermediate or resistant to antibiotics in accordance with current Clinical Laboratory Standard Institute (CLSI) recommendation. Maximum sample was resistance (≤11/≤12) tothe multiple antibiotics like, Ampicillin, Amoxiclav, Cefotaxime, Co-Trimoxazole, Gentamicin, Tobramycin etc. ExceptMeropenem (**Table 1)** [3,6]

Test/ Report Group	**Antimicrobial agent**	**Disk Content**	**Interpretive Categories & Zone Diameter Breakpoints (nearest whole mm)**		
			S	**I**	**R**
B-Lactam Inhibitor Combinations	Ampicillin-Sulbactam	10/10 µg	≥15	12-14	≤11
,,	Amoxiclav	10/10 µg	≥15	12-14	≤11
Cephems	Cefotaxime	30µg	≥23	15-22	≤ 14
Fluoroquinolones	Co-Trimoxazole	5/10 µg	≥21	16-20	≤14
Aminoglycosides	Gentamicin	10 µg	≥15	13-14	≤12
,,	Tobramycin	10 µg	≥15	13-14	≤12
Carbapenem	Meropenem	10 µg	≥ 18	15 - 17	≤ 14

Table 1: - **Results of the multiple antibiotics sensitivity test according to the order as the experiment Were performed.**

Percentage of Inhibition: -The optical density of the A.baumannii under the influence of homoeopathic preparation. All data of optical density were demonstrated in (Table 2& Graphical presentation) mean of triplicate with standard deviation (One-way ANOVA).

HOMOEOPATHIC MEDICINES	**POTENCY**	**OPTICAL DENSITY AGAINST A.baumanni**	**PERCENTAGE (%) INHIBITION**
BRONIA ALBA	6CH	0.115 ± 0.005	39%
	12CH	0.154 ± 0.011	54%
	30CH	0.237 ± 0.05	70%
	200CH	0.243 ± 0.056	71%
	1M	0.351 ± 0.047	80%
	10M	0.384 ± 0.012	82%
	MT	0.578 ± 0.078	82%
	VC	0.705 ± 0.058	0%
	PC	0.154 ±0.008	54%

BELLADONNA	6CH	0.144 ± 0.003	45%
	12CH	0.151 ± 0.005	47%
	30CH	0.148 ± 0.005	46%
	200CH	0.148 ± 0.004	46%
	1M	0.160 ± 0.008	50%
	10M	0.132 ± 0.006	40%
	MT	0.486 ± 0.021	84%
	VC	0.793 ± 0.079	0%
	PC	0.190 ± 0.006	58%
LACHESIS MUTUS	6CH	0.130 ± 0.014	40%
	12CH	0.148 ± 0.006	48%
	30CH	0.176 ± 0.006	56%
	200CH	0.197 ± 0.005	61%
	1M	0.177 ± 0.009	56%
	10M	0.234 ± 0.029	67%
	VC	0.776 ± 0.111	0%
	PC	0.155 ± 0.011	50%

Table 2: - The optical density with percentage of inhibition of the A.baumannii under the influence of homoeopathic preparation& control.

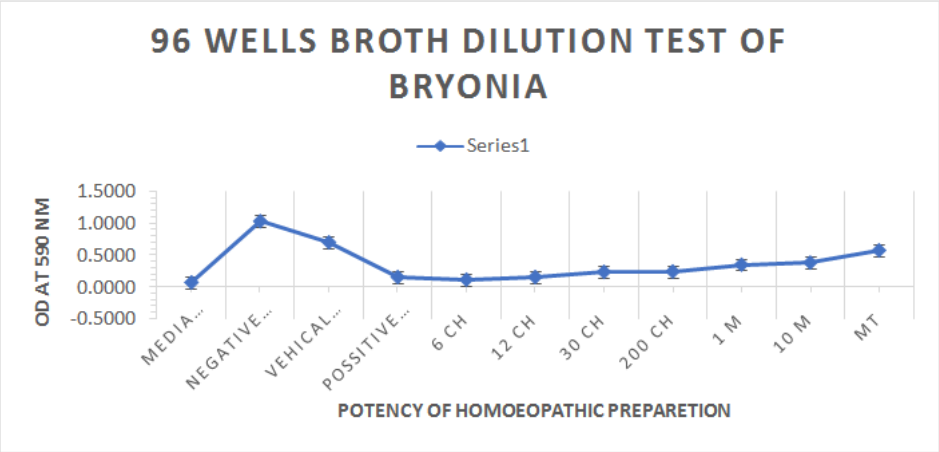

Figure 1: -Growth patternof an *Acinetobacter baumannii*in presence of*Bryonia alba*.

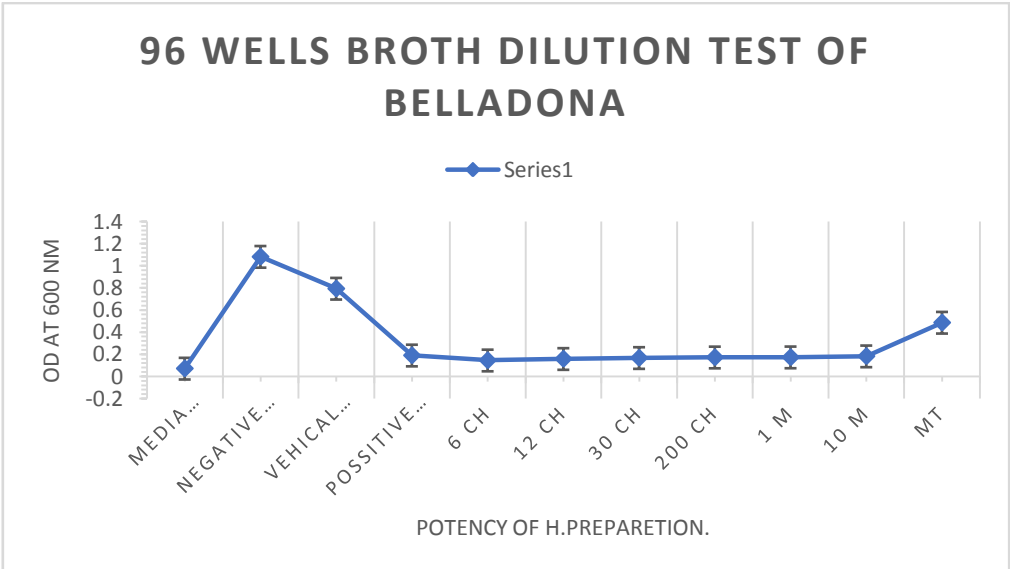

Figure 2: - Growth patternof an *Acinetobacter baumannii*in presence of*Belladona*

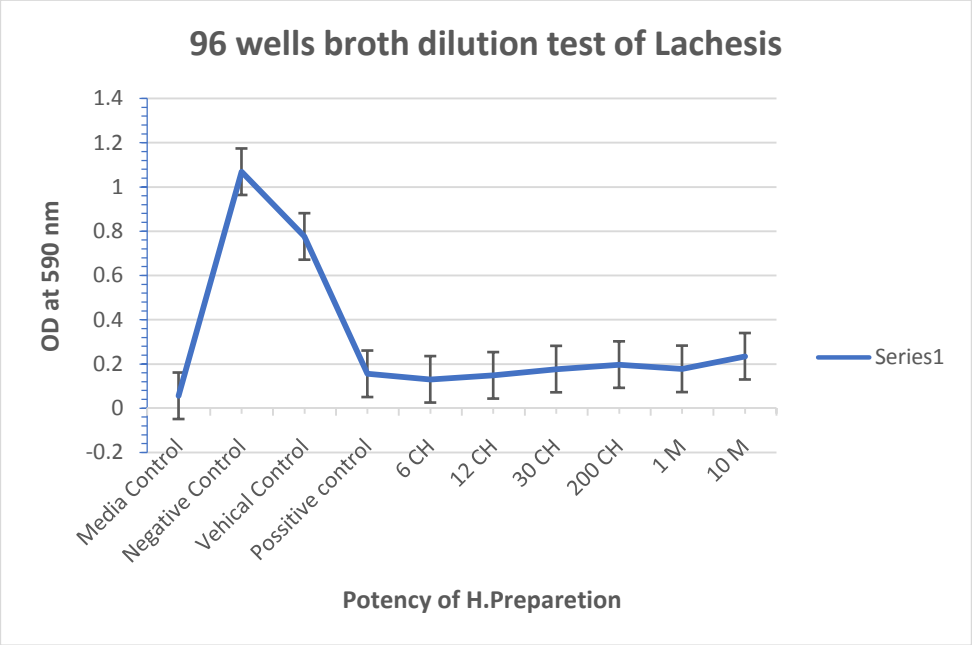

Figure 2: -Growth patterns of an *Acinetobacter baumannii*in Presence of*Lachesis mutus*.

Agar Well Diffusion Method/Ditch (punch well) Method: -

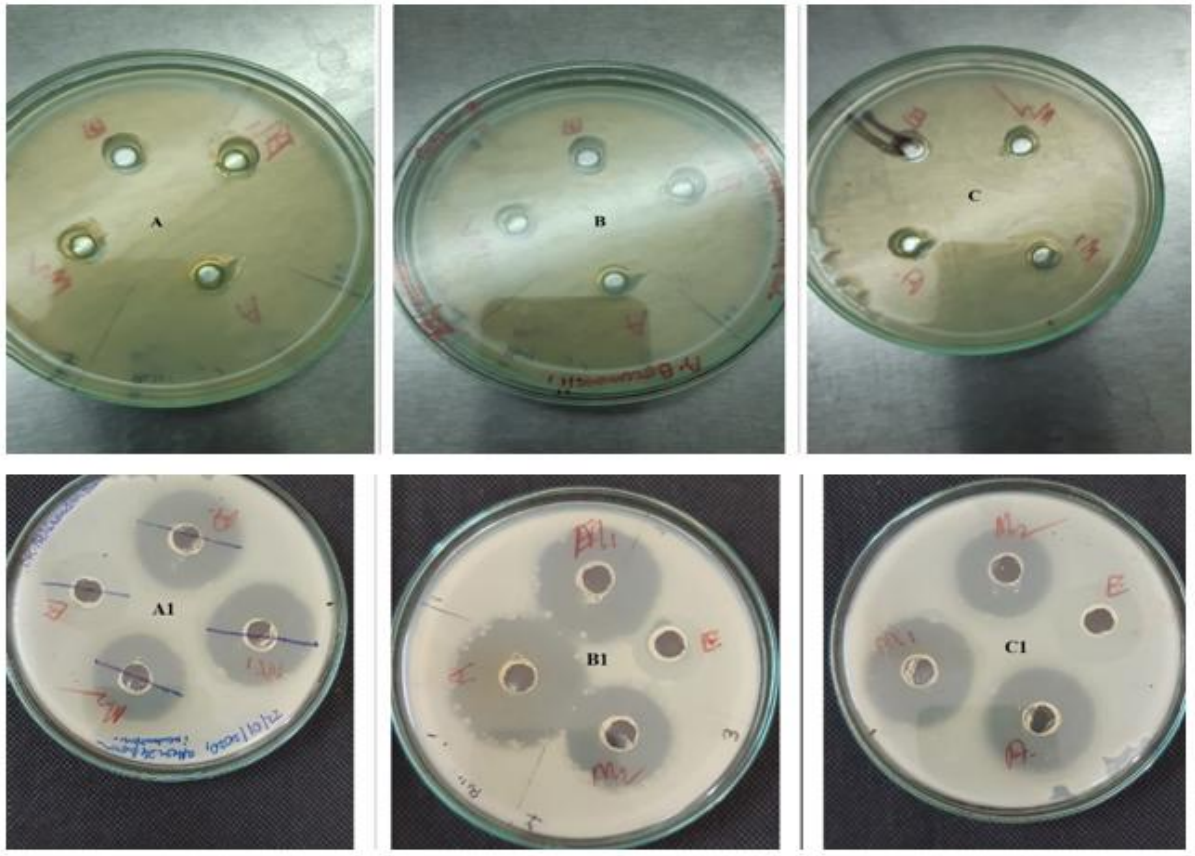

Figure 1: Anti-bacterial assay of Homoeopathic medicines & Control (M1- 6 CHmedicine, M2- 200 CH, E- Vehicle control, A- Positive Control) by Ager Well diffusion method.

Table 3: - Zone of inhibition of homoeopathic preparation & controls byAger well- Diffusion Assay (Mean and Standard Deviation)

Medicine Name	Zone of Inhibition (Mean ± Standard Deviation) in c.m		Positive Control	Vehicle Control
	6 CH	200 CH		
Bryonia alba	**15 ± 7.055**	**14 ± 6.906**	**15 ± 7.34**	**11 ± 5.42**
Belladonna	**12 ± 6.65**	**13.66 ± 7.57**	**12 ± 7.23**	**16 ± 9.86**
Lachesis Mutus	**15.33 ± 8.96**	**14.33 ± 8.66**	**11 ± 6.65**	**16 ± 9.53**

IV. Discussion:

Today's Health sector is very much famous due to infectious Disease. Hospital is the main source of infection, which through spread lots of gram-negative & gram-positive bacteria. As per W.H.O criteria they were categorised all MDR bacteria's in 3 point like, Critical, High & Medium. *Acinetobacter baumannii* is the 1st position/ mention on this Priority-1 (critical)category. [1,5,15]*Acinetobacter baumannii* is very severe problem in the hospital due its Nosocomial infection & it is multi drug resistance ability [1,7,9]. Almost all isolated samples of pathological lab. is very much present to these gram-negative bacteria. On these Policies antibiotics have limited success. Homeopathy may have a role to play in combating the development of antibiotic resistance [5,7]. Homoeopathic preparation like *Bryonia, Belladona& Lachesis* are covered almost all indication of Acinetobacter baumanniiproducedsymptoms [12]. This Homoeopathic preparation were selected by the combining of all the symptoms of A.baumannii through Murphy Repertory&Reverified by Herring Guiding symptoms [12].Meropenem is used as a broad spectrum antibiotic from Carbapenem group for Positive control.This experimental study was not done before in Homoeopathy, According to our survey. These experiments were performed to see the antibacterial effect of homoeopathic preparations against A. baumannii&also seen which potencies is very sensitive against A.baumannii by the performed an In-vitro study.The present study plane was done by wells diffusion method using of 96 microwell titter plates. This is gave very good results against the A.baumannii. *Bryonia 6CH****(39% growth,*****0.115 ± 0.005)***& *Lachesis 6CH****(*****40% growth,0.130 ± 0.014)** have shown very good sensitive result irrespective of other potencies along with Belladonna.

The system of Homoeopathic medicine works on the principal of dynamization which is increased our immune systems& it is fight against the pathogens. Homoeopathy is a demonstrably effective treatment option for a range of human infectious diseases. Homoeopathic treatment can be at least equivalent in effectiveness to antibiotics for certain human infectious diseases.

A.baumanniihasa MDR character&we were going to study Kirby-Bauer process. Bacteria were classified as susceptible, intermediate or resistant to antibiotics in accordance with current Clinical Laboratory Standard Institute (CLSI) recommendation. Maximum sample was resistance (≤11/≤12) to the multiple antibiotics like, Ampicillin, Amoxiclav, Cefotaxime, Co-Trimoxazole, Gentamicin, Tobramycin etc. Except Meropenem.

The antagonistic effects observed with homoeopathic medicines is almost equivalent to the meropenem sensitivity. Meropenem has been recorded showing resistance against some isolated culture of A.boumannii with they produced lots of adverse drug reaction to theindividual. Homoeopathy shows no adverse drug reaction to the individual. Results of disk diffusion expels as compared to modern medicine confirmed 6CH potency of *Bryonia (***15 ± 7.055)***&Lachesis*(**15.33 ± 8.96)** as an effective measures against *A.baumannii***(p<0.005).** The results indicate inhibited potencies of homoeopathic medicine against *A.baumannii* that would be helping in prevents nosocomial spread.

Today modern world homoeopathic system of medicine proving their effect based on evidence. The antagonistic anti-bacterial effect of the Bryonia, Belladonna& Lachesis were almost proved that homoeopathic medicines have some efficacy to prevents A. boumannii'snosocomial infection.The claim can be substantiated further by In-vivo experimentation for mode of action of selected medicines.

Conclusion: -In this study *Bryonia, Belladona& Lachesis* was interfering in the metabolism of the A.baumannii. Specially *Bryonia& Lachesis 6CH* potencies have really good results against A.baumannii.In modern medicine like,Ampicillin, Amoxiclav, Cefotaxime, Co-Trimoxazole, Gentamicin, Tobramycin etc. Except Meropenem are sensitive against A.boumannii. Meropenem have some adverse drug reaction whereas homoeopathic medicine has no any side effect. Results of disk diffusion expels as compared to modern medicine confirmed 6CH potency of *Bryonia (***15 ± 7.055)***& Lachesis* (**15.33 ± 8.96)** as an effective measures against

*A.baumannii***(p<0.005).** The results indicate inhibited potencies of homoeopathic medicine against *A.baumannii* that would be helping in prevents nosocomial spread. This result of that experiments supports that concept of "evidence-based medicine" in Homoeopathy. In future Homoeopathic medicine should be used in nosocomial infection cases. The claim can be substantiated further by In-vivo experimentation.

Acknowledgments

The author wish to thanks Bharati Vidyapeeth(Deemed to be) Homoeopathic Medical College & Hospital, Bharati Vidyapeeth(Deemed to be)Medical College & Hospital(Microbiology depot.), Bharati Vidyapeeth(Deemed to be)Rajiv Gandhi institute of Information Technology& Biotechnology for providing the laboratory facilities. Mrs. Anita Patil, PG Co-coordinator of B.V.D.U.H.M.C, Dr.R. Valavan, Scientific Officer Dr.Willmarschwabeindia Pvt Ltd,Noida, Utter Pradesh.. I would also like to thank my parents for providing financial support.

FinancialSupport: -This research received no specific grant from any funding agency in the public, commercial or not-for-profit-sectors.

Conflict of Interest: -Neither the author nor the co-author has received any financial or non-financial help from any other source.

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Abhinandan Das, et. al. "Antagonistic Effect of Homoeopathic Preparations on Acinetobacter baumannii, A Common Nosocomial Infections Agent through In Vitro Study."*IOSR Journal of Nursing and Health Science (IOSR-JNHS)*, 9(3), 2020, pp. 36-42.