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

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Abstract
Background: Acinetobacter baumannii is 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 of Multi Drug Resistance (MDR). Homoeopathy may have a role to play in combating the development of anti-biotic resistance.

Materials and Methods: Clinically Isolated Samples were collected from Bharati Vidyapeeth 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 pathogen A. 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 & Lachesis shows 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 resistant (≤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. baumannii that 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.


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I. Introduction
Infectious diseases are very common in developing countries due to environmental changes, polluted soil, 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, prolonged hospital 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].

Recent 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 coccobiacillus [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, medical devices, equipment, Hand sanitizers, dispensary’s etc. not only that, they have the capacity to survive for prolonged periods on inanimate objects.[2] *Acinetobacter baumannii* is an important cause of nosocomial infection/ Hospital acquired infection. It is produced bacteremia, pneumonia, ventilator -associated pneumonia, meningitis, urinary tract infection, catheter- associated UTI, central line- associated bloodstream infection, Wound infection [5,7,8,9].

Homeopathic 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 of antibiotic resistance. Homeopathic preparation like Bryonia, Belladona & Lachesis are covered all indication of *Acinetobacter baumannii*produced symptoms. 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 fulfillment against MDRA.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 Homeopathic pharmacopoeia of India Vol-1.

Sample Culture: -All clinically isolated culture of *Acinetobacter baumannii* 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 Vidyapeeth Hospital, 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+20µl 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 Δo at 37°C O.D was measured at 600nm.

Antibiotic sensitivity/Kirby -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. [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 margin 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/Triple value. Mean & Standard 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 were obtained 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) to the multiple antibiotics like, Ampicillin, Amoxiclav, Cefotaxime, Co-Trimoxazole, Gentamicin, Tobramycin etc. Except Meropenem (Table 1) [3,6]

<table>
<thead>
<tr>
<th>Test/ Report Group</th>
<th>Antimicrobial agent</th>
<th>Diameter Breakpoints (nearest whole mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
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<tr>
<td>B-Lactam Inhibitor Combinations</td>
<td>Ampicillin- Sulbactam</td>
<td>10/10 µg</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Cefotaxime</td>
<td>30 µg</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Co-Trimoxazole</td>
<td>5/10 µg</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Gentamicin</td>
<td>10 µg</td>
</tr>
<tr>
<td>Carapenem</td>
<td>Meropenem</td>
<td>10 µg</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>6CH</th>
<th>12CH</th>
<th>30CH</th>
<th>200CH</th>
<th>1M</th>
<th>10M</th>
<th>MT</th>
<th>VC</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BELLADONNA</td>
<td>0.144 ± 0.003</td>
<td>0.151 ± 0.005</td>
<td>0.148 ± 0.005</td>
<td>0.148 ± 0.004</td>
<td>0.160 ± 0.008</td>
<td>0.132 ± 0.006</td>
<td>0.486 ± 0.021</td>
<td>0.793 ± 0.079</td>
<td>0.190 ± 0.006</td>
</tr>
<tr>
<td>LACHESIS MUTUS</td>
<td>0.130 ± 0.014</td>
<td>0.148 ± 0.006</td>
<td>0.176 ± 0.006</td>
<td>0.197 ± 0.005</td>
<td>0.177 ± 0.009</td>
<td>0.234 ± 0.029</td>
<td>0.736 ± 0.111</td>
<td>0.155 ± 0.011</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Growth pattern of an Acinetobacter baumannii in presence of Bryonia alba.

Figure 2: Growth pattern of an Acinetobacter baumannii in presence of Belladona.
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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 by Agar well- Diffusion Assay (Mean and Standard Deviation)**

<table>
<thead>
<tr>
<th>Medicine Name</th>
<th>Zone of Inhibition (Mean ± Standard Deviation) in cm</th>
<th>Positive Control</th>
<th>Vehicle Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 CH</td>
<td>200 CH</td>
<td>15 ± 7.055</td>
</tr>
<tr>
<td>Bryonia alba</td>
<td></td>
<td></td>
<td>12 ± 6.65</td>
</tr>
<tr>
<td>Belladonna</td>
<td></td>
<td></td>
<td>15.33 ± 8.96</td>
</tr>
</tbody>
</table>

**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. [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. Homoeopathy may have a role to play in combating the development of antibiotic resistance [5,7]. Homoeopathic preparation like Bryonia, Belladonna & Lachesis are covered almost all indication of Acinetobacter baumanniproducedsymptoms [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. baumannii has a 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. baumannii with they produced lots of adverse drug reaction to the individual. 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. baumannii nosocomial infection. The claim can be substantiated further by In-vitro experimentation for mode of action of selected medicines.

**Conclusion:** - In this study Bryonia, Belladonna & 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. baumannii. Meropenem have some adverse drug reaction whereas homoeopathic medicine has no any side effect. Results of disk diffussion expels as compared to modern medicine confirmed 6CH potency of Bryonia (15 ± 7.055) & Lachesis (15.33 ± 8.96) as an effective measures against...
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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.

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