Chapter 5

Significant and Findings
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1. *Vigna aconitifolia* sprouts broth (5%) containing 0.5% NaCl and 1.5% agar was the best VEG-base medium as compared to the two other seeds and sprouts in this study and the optimum growth of different bacteria was observed on this medium similar on Nutrient agar.

2. Chemical analysis revealed that *Vigna aconitifolia* sprouts contained reducing sugars (glucose and galactose), different elements and proteins as the most important essential compositions for growth of bacteria.

3. Chopade-Kazemi VEG (CK) medium was prepared by 5% of *Vigna aconitifolia* VEG-base medium with NaCl (5 g/lit), ox bile (5g/lit), crystal violet (0.032g/lit) and bacteriological Agar 15 (g/lit) which made it differential and selective for identification of *Acinetobacter* strains. This medium was more differential than VRBA for identification of *Acinetobacter* strains as their colonies were dark blue. CK is the best and reliable differential medium for isolation and identification of *Acinetobacter* species.

4. VEG-Holton’s medium was prepared by 5% of extract of *Vigna aconitifolia* sprouts, 0.5% Saccharose, 0.5% Fructose, 0.5% Maltose, 0.002% Phenol red, 0.5% ox bile and 1.5% agar. Bacterial growth and differential activity of VEG-Holton’s medium was similar to the Holton’s medium by observing pink colonies of *Acinetobacter* strains.

5. Both CK and VEG-Holton’s medium are economical because of their VEG- base source.

6. After sampling from UTI and urinary catheters, twenty eight tentative *Acinetobacter* spp. were obtained from preliminary tests and all of them were confirmed as a member of *Acinetobacter* spp. by chromosomal DNA transformation assay. Twenty two *Acinetobacter* strains isolated and identified
from UTI (obtained from one of the previous studies in our lab) were used in this study.

7. Analytical profile index (API) ID32 GN assay for all tentative Acinetobacter spp. revealed that they were forty four A. baumannii and three A. lwoffii from UTI and the rest isolates were A. baumannii from urinary catheters.

8. Antibiotic disc susceptibility test was performed by use of thirty nine antibiotics which covers all major class of antibiotics used in UTI. More than 70% of Acinetobacter strains were highly resistant to cephalosporins, beta lactams, quinolones and aminoglycosides but 98% Acinetobacters were sensitive to Colistin.

9. MICs of fifteen antibiotics were determined against fifty Acinetobacter strains. MICs of antibiotics from all groups, except colistin and imipenem were >512 µg/ml against 91% A.baumannii strains. MICs of thirteen antibiotics except ceplopazone and nitrofurantion against all A. lwoffii strains were <128 µg/ml.

10. MICs of Acinetobacter strains showed 14% multi drug resistant (MDR), 78% pan drug resistant (PDR) and 8% extreme drug resistant (XDR).

11. All strains of A.baumannii showed positive activity for β–lactamase enzyme by qualitative determination but A.lwoffii strains did not show any activity.

12. Six A. baumannii strains were determined with strong hydrophobicity index (70-79.4%) and were selected for further studies.

13. Quantitative and qualitative analysis about six A. baumannii on glass and polypropylene tubes showed that shaking conditions were suitable for biofilm formation. On the other hand, all six tested A. baumannii strains formed denser aggregates on polypropylene tubes than on the glass surfaces.
14. Light microscopic examinations showed that cells of *A. baumannii* on polycarbonate surfaces produced more biofilm aggregates than on glass. Similar results were obtained when *A. baumannii* biofilms were examined through epifluorescence microscopy and phase contrast microscopy.

15. By SEM, stacks of *Acinetobacter* cells on glass, polycarbonate plate and urinary catheters were observed.

16. Maximum biofilm formation by six *A. baumannii* was observed at 30°C, pH 7 and 5.0 g l⁻¹ NaCl.

17. All of the six *A. baumannii* biofilm formers showed positive reactions with human erythrocytes (lectin activity). These observations are quite significant since lectin leading to intracellular adhesion and biofilm accumulation.

18. Significant reductions was observed in the number of the six tested biofilm forming *Acinetobacter* strains adhered to the catheter when treated with sub-minimum inhibitory concentrations of colistin (0.5 and 1 μg/ml MIC), confirming that colistin interferes with the adhesion properties of the tested bacterial strains.

19. Twenty five *A. baumannii* and three *A. lwofii* harbored single or multiple plasmids and molecular size of them ranged from 1.7 to 56.12 Kb.

20. Plasmids pUPI 802, pUPI 804 to pUPI 807 (Ci ⁵) were cured from *A. baumannii* A3 by Plumbagin with curing efficiencies of 4.5 per cent.

21. Plasmids pUPI 803 to pUPI 805 (Cp⁵,A⁵,Nf⁵) were intergeneric conjugally transferred from *A. baumannii* A3 to *E.coli* HB 101 at frequency 1.5×10⁻⁷ transconjugants per recipient cell count.
22. Plasmid pUPI 806 (Cs\(^r\), Cp\(^r\)) was transferred from *A. baumannii* A3 to *A. baylyi* 7054 trpE by transformation and frequency of transformation was \(2.9 \times 10^3\) transformants/µg plasmid DNA.

- Overall, this study indicates that CK VEG and VEG-Holton’s media are two new veg-base media with selective and differential activity for isolation of *Acinetobacter* strains from clinical and environmental sources. Antibiotic resistance; biofilm formation of *Acinetobacter* strains isolated from UTI revealed that *Acinetobacter* strains can transfer naturally occurring plasmids. The present study shows that clinical isolates of *A. baumannii* contained multi drug resistant plasmids and they had mostly the ability to form biofilm in abiotic surfaces such as urinary catheters. Biofilm formation as well as the potential of spreading the antibiotic resistant plasmids to the other pathogens was the important mechanism in the pathogenicity of these bacteria.