INTRODUCTION
Biofilm formation by bacterial pathogens is an increasing cause of morbidity and mortality associated with chronic and nosocomial infections. Biofilms are defined as adherence of bacterial cells protected by a self-synthesized extra polymeric substance (EPS). Biofilms are very difficult to eradicate and cause recurrent infections. Biofilm formation has been linked to the survival of pathogenic bacteria in the hospital environment and it has been connected to infections associated with indwelling medical devices. The members of the genus Acinetobacter are ubiquitous Gram-negative cocobacilli that are frequently found in the environment but also in the hospital setting where they have been associated with outbreaks of nosocomial infections. A. baumannii has emerged as the most common pathogenic species involved in hospital-acquired infections; this multi drug resistant (MDR) opportunistic pathogen can survive on nutrient-limited surfaces for several days, even in dry conditions and in the harsh hospital environment. One reason for this emergence may be its persistence in the hospital wards, in particular in the intensive care unit; this persistence could be partially explained by the capacity of these microorganisms to form biofilm. Antibiotic resistance is often associated with infection and is therefore also related to virulence, as in the cases of biofilm producing microorganisms or intracellular infections. Management of multidrug-resistant Acinetobacter spp. infections is a great challenge for physicians and clinical microbiologists. Its ability to survive in a hospital setup and its ability to persist for extended periods of time on surfaces makes it a frequent cause for healthcare-associated infections and it has led to multiple outbreaks. Recent studies have shown that certain bacterial species secrete extracellular products that inhibit the settlement of potential competitors. Bacterial extracellular polysaccharides have been shown to mediate many of the cell-to-cell and cell-to-surface interactions that are required for the formation, cohesion and stabilization of bacterial biofilms. However, recent studies have identified several
bacterial polysaccharides that inhibit biofilm formation by a wide-spectrum of bacteria and fungi both in vitro and in vivo.[7] This study was aimed to isolate the MDR and Non MDR Acinetobacter species from different clinical samples and to check their ability to form biofilm and the effect of the extracted Exopolysaccharide of Pseudomonas aeruginosa on the biofilm formation on these MDR and Non MDR strains of Acinetobacter species.

MATERIAL AND METHODS
Study design: Prospective study
Setting: This study was conducted at Department of Microbiology, Yenepoya Medical College, Mangalore

METHODS OF MEASUREMENT
Inclusion criteria: Acinetobacter species isolated from all clinical samples.
Exclusion criteria: Acinetobacter species isolated from the patients who are on prior antibiotic treatment.

1. Strain collection
In vitro reported MDR and Non-MDR Acinetobacter species from diagnostic Microbiology laboratory was collected and further processed to check the biofilm formation and effect of exopolysaccharide on it.

2. Biofilm formation assay
Isolates from fresh agar plates were inoculated in brain heart infusion (BHI) with 2% sucrose and incubated for 18 hour at 37°C and diluted 1 in 100 with fresh medium. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plate wells were filled with 0.2 ml aliquots of the diluted cultures and only broth served as control to check sterility and non-specific binding of media. The tissue culture plates were incubated for 24 hours at 37°C. After incubation content of each well was gently removed by tapping the plates. The wells were washed four times with 0.2 mL of phosphate buffer saline (PBS pH 7.2) to remove free-floating planktonic bacteria. Biofilms formed by adherent organisms in plate were fixed with sodium acetate (2%) and stained with crystal violet (0.1% w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent cells usually formed biofilm on all side wells and were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria were determined with a micro ELISA reader at wavelength of 570 nm (OD570 nm) and interpreted as weak, moderate and high biofilm producers if OD values <0.120, 0.120-0.240 and >0.240 respectively.[8]

3. Biofilm inhibition assay
Biofilm inhibition assay was done by using extracted Exopolysaccharide according to Pawar ST et al (2013). [9] Exopolysaccharide of Pseudomonas aeruginosa was extracted by the Ethanol precipitation method and used for biofilm inhibition.

Statistical methods used: Descriptive statics was used for the analysis of the data. Proportion was used to study the biofilm formation and inhibition among MDR and Non MDR Acinetobacter species.

Ethical guidelines followed by the investigators: This study was conducted after obtaining the permission from the Institutional Ethics Committee.

RESULTS AND DISCUSSION
In our study total 58 Acinetobacter species obtained, out of which 39 (67.2%) were MDR and 19 (32.8%) were Non MDR. Strains obtained from various clinical samples like- 16 pus (11 MDR and 4 Non-MDR), 13 ET-aspirate (10 MDR and 3 Non-MDR), 10 urine (6 MDR and 4 Non-MDR), 13 sputum (8 MDR and 5 Non-MDR) and 7 blood (4 MDR and 3 Non-MDR) as shown in Table 1.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of MDR isolates (n=39)</th>
<th>Number of non-MDR isolates (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>11 (28.2%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>ET aspirates</td>
<td>10 (25.6)</td>
<td>3 (15.8%)</td>
</tr>
<tr>
<td>Urine</td>
<td>6 (15.4%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Sputum</td>
<td>8 (20.5%)</td>
<td>5 (26.4%)</td>
</tr>
<tr>
<td>Blood</td>
<td>4 (10.3%)</td>
<td>3 (15.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (100%)</td>
<td>19 (100%)</td>
</tr>
</tbody>
</table>

Out of 39 MDR biofilm producers, 16 (41.1%) were inhibited by the effect of exopolysaccharide of P.aeruginosa and 23 (58.9%) isolates were not inhibited by the effect of exopolysaccharide. Whereas, out of 19 Non MDR biofilm producers, 12 (63.2%) were inhibited by the effect of exopolysaccharide and 7 (36.8%) isolates were not inhibited by the effect of exopolysaccharide as shown in Table 2.

DISCUSSION
Acinetobacter species is found to be non-pathogen in healthy individual whereas generally responsible for opportunistic infections. The risk of acquisition of Acinetobacter infection depends upon risk factors like prolonged hospital stay, receipt of invasive medical devices, prolonged exposure to antimicrobial agents, surgical and invasive procedures and severe underline...
conditions like diabetes. Antimicrobial drug resistance is raising among Acinetobacter species since past decades[10] therefore management of MDR Acinetobacter species infection is a great challenge for physician and clinical microbiologist.[5]

Shivranjani V et al., (2013)[11] have stated that more isolation of Acinetobacter species was from pus which was 38.5% followed by endotracheal aspirate was 20.49%, urine 19.67% and blood was 5.7%. However in our study we have also found that the more isolation of MDR Acinetobacter species was from pus (28.2%) followed by endotracheal aspirate (25.6%) followed by sputum (20.5%) which is in contrary to the Shivranjani et al., in which the third commonest isolation was from urine sample followed by urine (15.4%) and blood (10.3%). However in our study in case of Non-MDR Acinetobacter spp. more isolation was found to be from sputum (26.4%) followed by pus and urine (21%) followed by endotracheal aspirate and blood (15.8%). Whereas another study done by Mastofi et al., (2011)[12] showed the highest isolates from blood samples.

In our study, out of 58 isolates 39 (67.2%) were MDR and 19 (32.8%) were Non-MDR. Similarly Sivaranjani V et al., (2013) has also reported the emergence of MDR Acinetobacter spp. from various clinical samples from south India.[11]

Acinetobacter species have a very strong capability to produce biofilm and they can acquire antibiotic resistance to multiple drugs[11] and we have also found similar results in our study in which, out of 39 MDR Acinetobacter spp., 16 (41.1%), 2 (5.1%), 21 (53.8%) were strong, moderate and weak biofilm producers respectively. However out of 19 Non-MDR Acinetobacter species 11(57.9%), 2 (10.5%) and 6 (31.6%) were strong, moderate and weak biofilm producers respectively.

In accordance to our study Zhiqiang Qin et al., (2009)[6] also have reported that the Acinetobacter biofilms get disrupted by exopolysaccharide secreted by P. aeruginosa. In our study, we found that P. aeruginosa inhibits biofilms in an in-vitro microtitre plate assay. Among 39 MDR isolates 16(41.1%) were inhibited by exopolysaccharide, whereas 23 (58.9%) was not inhibited and in case of 19 Non-MDR isolates, 12 (63.2%) was inhibited and 7(36.8%) was not inhibited.

CONCLUSION
This study has shown that P. aeruginosa O1 strain expresses extracellular polysaccharides which have a better inhibitory effect on Non MDR biofilm producing Acinetobacter spp. compared with MDR biofilm producers. In this study, we found that P. aeruginosa PO1 strain produces and extracellular exopolysaccharide that inhibits biofilm formation in phylogenetically diverse bacteria.

Conflicts of Interest: Nil

ACKNOWLEDGEMENT
I would like to thank Department of Microbiology, Yenepoya Medical College for providing support to undertake this study.

REFERENCES