ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 02 | February - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

BIOFILM PRODUCTION AND ITS ASSOCIATION WITH MULTIDRUG RESISTANCE AMONG THE CLINICAL ISOLATES OF *ACINETOBACTER* SPECIES IN A TERTIARY CARE CENTRE

Microbiology

Dr. Arundathi. H. A* Assistant professor, Department Of Microbiology, Karwar Institute Of Medical Sciences, Karwar. *Corresponding Author

Dr. Mallikarjun Koppad Associate Professor, Department of Microbiology, Shimoga Institute Of Medical Sciences(SIMS), Shimoga.

Dr. Prakash N Assistant Professor, Department Of Microbiology, SIMS, Shimoga.

ABSTRACT

Background: *Acinetobacter*, a known nosocomial pathogen has raised concern due to its multi drug resistant(MDR) nature. Though its resistance is attributed to various factors, its ability to produce biofilm is said to be the important one. Hence, an attempt is made here to detect biofilm production and its association with multi drug resistance among the clinical isolates of Acinetobacter. **Materials And Method:** A total of 100 *Acinetobacter* species isolated in the Clinical samples received at our laboratory during Jan-Dec 2015 was subjected for the study. Antimicrobial susceptibility testing was performed as per CLSI guidelines. Biofilm production was detected by tube method and microtitre plate method. **Result:** 69(69%) out of total 100 *Acinetobacter* species were *A.baumannii*, the most important pathogen of the genus. 87(87%) isolates showed multi-drug resistance. 42(42%) isolates showed biofilm production by tube method and 69(69%) showed positive result with microtitre plate method. And it was found that all biofilm producers were multi drug resistant. **Conclusion:** The study gives a clear picture of extremely significant association between biofilm production and multi drug resistance among *Acinetobacter* species. Hence, measures targeted towards reduction of biofilm formation by the organism are a welcome move to control raising cases of multi drug resistant *Acinetobacter* infections.

KEYWORDS

Acinetobacter, Acinetobacter baumannii, MDR, biofilm, microtitre plate method

INTRODUCTION

Acinetobacter are known cause of nosocomial infections mainly associated with surgeries and usage of artificial devices. Adhesion of bacteria to host cell facilitate colonization followed by infection. Production of biofilm is considered as one of the important pathogenic feature.[1,2]

Various mechanisms are described which attribute to antibiotic resistance observed in strains which produce biofilm. These include: altered/reduced antibiotic penetration, variation in rate of growth of the bacteria, [3]ability to increase mutation rate, an increased expression of efflux pump. [4]Formation of this biofilm, which is a complex aggregation of microorganisms, wherein the cells are embedded in a self-produced matrix of extracellular polymeric substance (EPS) is linked with MDR among the organism.

Hence an attempt is made to study the association of biofilm production with multi drug resistance so as to stress on the importance of using biofilm inhibition strategies wherever necessary.

MATERIALS AND METHODS

All clinical samples received in our laboratory were included in this cross-sectional study. Ethical committee approval from the institution was taken.

100 *Acinetobacter* species isolated and speciated by conventional methods were included for the study. [5,6,7] AST was done by disc diffusion method as per CLSI guidelines.[8]

DETECTION OF BIOFILM FORMATION:

Biofilm production was estimated qualitatively for all isolates of *Acinetobacter* by tube method and microtitre plate method as described by Cristensen et al. [9,10,11]

Figure 1: Tube Method For Biofilm Detection, Showing Positive And Negative Results

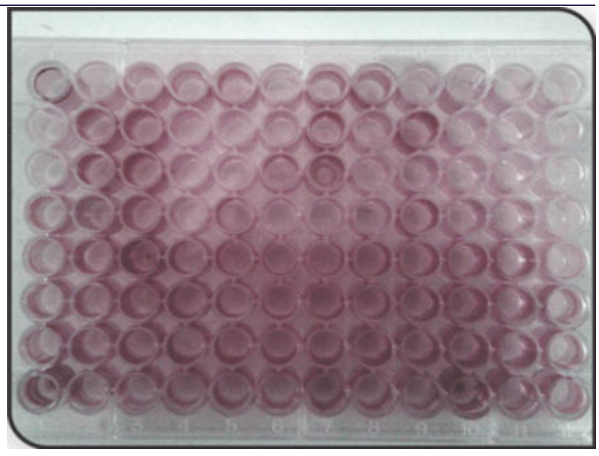

Figure 2: Detection of biofilm formation by *Acinetobacter* isolates by microtitre plate method

RESULTS

Out of 100 *Acinetobacter* species isolated, different species isolated were *A.baumannii* (69), *A. lwoffii* (17), *A.calcoaceticus* (08), *A. haemolyticus*(05) and *A.junii* (01).

Sensitivity pattern of the isolates to different antibiotics is as follows: Ampicillin/Sulbactam(17%), Ceftazidime(15%), Ciprofloxacin(30%), Imipenem(60%), Meropenem(67%), Gentamicin(17%), Amikacin(58%), Piperacillin-Tazobactam(11%), Cefipime(11%), Cefotaxime(18%), Ceftriaxone(12%), Co-trimoxazole(21%).

Out of 100 *Acinetobacter* isolates, 42(42%) isolates showed biofilm production by tube method and 69(69%) showed positive result with microtitre plate method. All isolates that showed positive result with microtitre plate method were considered as biofilm producers.

Resisitance to Ampicillin/Sulbactam (100% v/s 45.2%), Ceftazidime (100% v/s 51.6%) Ciprofloxacin (84.1% v/s 38.7%), Imipenem (46.4%v/s25.8%) Meropenem (40.6% v/s 16.1%) ,Gentamicin (89.9% v/s 67.7%), Amikacin (52.2% v/s 19.4%), Piperacillin-Tazobactam (100% v/s 64.5%), Cefipime (100% v/s 64.5%), Cefotaxime(100% v/s 38.7%) Ceftriaxone (100% v/s 61.3%),Cotrimoxazole (92.8% v/s 48.4%) were higher among biofilm producers compared to non producers.

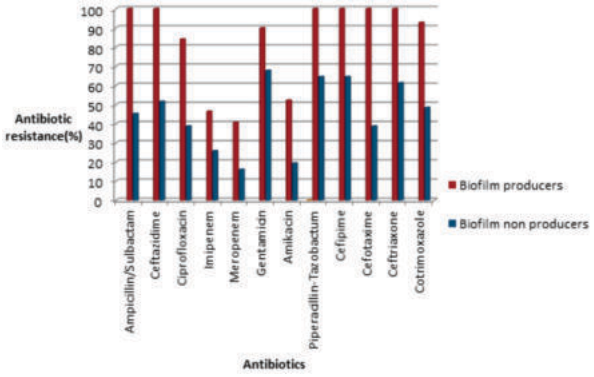

Chart 1: Resistance pattern of biofilm producing and non-producing *Acinetobacter* species

Isolates resistant to atleast three classes of antimicrobial agents were considered as Multidrug resistant (MDR). Association between biofilm production and MDR is depicted in chart 2.

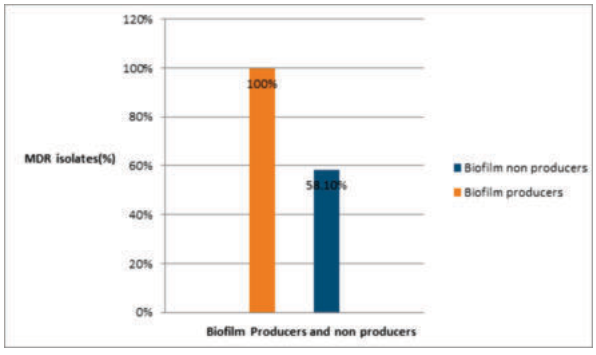

Chart 2 : Multi-drug resistance (MDR) among biofilm producing and non-producing *Acinetobacter* species

All biofilm producing isolates were multi-drug resistant ($p<0.0001$). Therefore, the association between biofilm production and multi-drug resistance among the *Acinetobacter* isolates is extremely statistically significant.

DISCUSSION

In the present study, out 100 *Acinetobacter* species were isolated, 59 were from blood, 31 were from pus samples and 10 were from urine samples. The majority of blood samples were from ICU, where out of 59 blood samples, 33(55.9%) were from NICU, 12(20.3%) were from PICU and 4(6.8%) from MICU. Thus, ICU samples accounted for 83.1% (49) of the blood samples and 49% (49) of the total samples.

Like many other studies on biofilm detection in bacteria, our study also showed better results with microtitre plate method as compared to tube method for biofilm detection.[12,13]

In the present study, biofilm production among *Acinetobacter* species was detected in 69% of the isolates. This is in line with the results of most of the studies Rodriguez Bano J et al[14], Nahar A et al[15], Marti S et al[16], Tripathi CP et al[17]who have established 63%,75%,63% and 62 % biofilm producers respectively.

In our study, 100% resistance was observed among the biofilm producers to β-lactam-β-lactamase inhibitor combination and to cephalosporins. A similar finding of 100% resistance to these two groups of antibiotics was observed in studies of Nahar A et al[15] and Kinikar A et al[4].

Highest sensitivity was noted with Carbapenems(60%-67%) followed by Aminoglycosides (58%).This pattern of resistance is not in line with study by Dheepa et al[10], Nahar A et al[15], Gurung J et al [3] where all have reported more than 50% resistance to carbapenems and aminoglycosides.

However the same pattern of resistance was noted with study by Tripathi et al [17]and Rodriguez-Bano J et al[14] where both the studies have reported maximum sensitivity to carbapenems followed by aminoglycosides.

Correlation between biofilm production and multidrug resistance in *Acinetobacter* species in various studies is as depicted in table 1.

Table 1: Biofilm Production And Its Association With Multi-drug Resistance Among *Acinetobacter* Isolates In Various Studies

Sl no.	Author	Year of study	Biofilm producing *Acinetobacter* isolates showing MDR (%)
1.	Rao et al[12]	2008	100
2.	Kinikar et al[4]	2013	100
3.	Nahar et al[15]	2013	100
4.	Gurung J et al[3]	2014	73
5.	Present study	2015	100

CONCLUSION

The study throws light on the current concern of biofilm production among the clinical isolates of *Acinetobacter* and its significant association with multi drug resistance. Attempts at implementation of biofilm inhibition strategies like use of phage therapy(where phages encoding biofilm degrading e enzymes are utilised), silver nanoparticles impregnated medical devices, cathelicides and lytic peptides, antimatrix agents, antiadhesin agents and chelating agents which is infections the significant are spreading rapidly in a hospital setting with the threat of emergence of resistance to the current therapeutic options. As the organism can survive well in hospital environment and acquire resistance at a faster rate, it becomes a very important task to put in all control measures like: Standard precautions, Environmental disinfection , Antimicrobial stewardship Program, Regular training programs on infection control policies, to be followed all the time to decrease spread of infection and emergence of MDR strains.

ACKNOWLEDGMENTS

I thank all my professors, colleagues and technicians who have helped me in carrying out my study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical committee clearance obtained.

REFERENCES

1) Joly-Guillou ML.Clinical impact and pathogenicity of *Acinetobacter*. Clin Microbiol Infect 2005; 11: 868–873.
2) Lee HW, Koh YM, Kim J, Lee JC, Lee YC, Seol SY et al. Capacity of multidrug resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. Clin Microbiol Infect. 2008; 14: 49-54.
3) Gurung J, Khyriem AB, Banik A, Lyngdoh WV, Choudhury B, Bhattacharyya P. Association of biofilm production with multidrug resistance among clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from intensive care unit. Indian Journal of Critical Care Medicine. 2013; 17(4): 214 - 218.
4) Kinikar A, Saini S, Bose S, Deorukhkar S. *Acinetobacter* Species an Emerging Nosocomial Pathogen: It`s Isolation pattern , Biofilm formation and Antibiotic Susceptibility profile. International Journal of Health Sciences and Research. 2014; 4(1): 79-86.
5) Fallon RJ, Young H. Neisseria, Moraxella, Acinetobacter. In. Collee JG, Fraser AG, Marmion BP, Simmons A." Mackie and McCartney Practical Medical Microbiology". Churchill Livingstone. 2006. 14th edition: 283-294.
6) Elliot Juni. 'Acinetobacter'.In. Brenner DJ, Krieg NR, Stanley JT. " Bergey's manual of systemic bacteriology". 2nd ed. Vol-2. USA. 425-437.
7) Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger et al. Gram negative non-fermenters. In. "Koneman's colour Atlas and Textbook of Diagnostic Microbiology". 6th ed. Philadelphia: Lippincott Williams and Wilkins Publishers; 2006: 353-355
8) Christensen GD, Bisno AL, Simpsom WA, Beachey EH. Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun 1982; 37:318-326.
9) Christensen GD, Simpson WA, Yonger JJ, Baddor LM, Barrett FF, Melton DM, Beachey EH. Adherence of Coagulase-negative *staphylococci* to plastic tissue culture plates: Aquantitative model for the adherence of Staphylococci to medical devices. J Clin Microbiol. 1985; 22: 996-1006.
10) Dheepa M, Rashme VL, Appalaraju B. Comparision of biofilm production and multiple

drug resistance in clinical isolates of *Acinetobacter baumannii* from a tertiary care hospital in South India. *Int J Pharm Biomed Sci.* 2011; 2(4): 103-107.
11) Pour NK, Devendra H, Prashant K, Dhakephalkar, Zamin FR, Zinjarde SS et al. Biofilm formation by *Acinetobacter baumannii* strains isolated from urinary tract infection and urinary catheters. FEMS Immunol Med Microbiol. 2011; 62:328-338.
12) Rao RS, Karthika RU, Singh SP, Shashikala P, Kanungo R, Jayachandran S et al. Correlation between biofilm production and multiple drug resistance in Imipenem resistant clinical isolates of *Acinetobacter baumannii*. Indian Journal of Medical Microbiology. 2008; 26(4): 333-7.
13) Mathur T, Singhal S, Khan S. Detection of biofilm formation among the clinical isolates of *Staphylococci*. Indian J Med Microbiol. 2006; 24: 25-29.
14) Rodriguez-Bano J, Marti S, Soto S, Fernandez-Cuenca F, Cisneros JM, Pachon J et al. Biofilm formation in *Acinetobacter baumannii*: associated features and clinical implications. European Society of Clinical Microbiology and Infectious Diseases. 2008; 14: 276-278.
15) Nahar A, Anwar S.Miah RA. Association of Biofilm formation with Antimicrobial Resistance among the *Acinetobacter* species in a Tertiary Care Hospital in Bangladesh. J medicine. 2013; 14: 28-32.
16) Marti S, Rodriguez-Bano J, Catel-Ferreira M, Jouenne T, Vila J, Seifert H. Biofilm formation at the solid-liquid and air-liquid interfaces by *Acinetobacter* species. BMC Res Notes. 2011; 4(5): 214-218.
17) Tripathi PC, Gajbhiye SR, Agarwal GN. Clinical and antimicrobial profile of *Acinetobacter* species: An emerging nosocomial superbug. Adv Biomed Res. 2014; 3 (13): 1-6.