Uttar Pradesh Journal of Zoology

Volume 45, Issue 9, Page 150-157, 2024; Article no.UPJOZ.3431
ISSN: 0256-971X (P)

Unravelling Antibiotic Resistance Profile and Biofilm Formation of *Acinetobacter baumannii* in Coimbatore Hospitals: A Comprehensive Study on Infection Management Strategies

Vidhya Vijaya Raghavan [a], T. Vishwanathan [b++*] and K. V. Ashok Raj [c#]

[a] Department of Microbiology, Research and Development Centre, Bharathiyar University, Coimbatore, Tamilnadu, 641046, India.
[b] Department of Microbiology, LRG Women's College, Tiruppur, Tamilnadu, 641604, India.
[c] PSG College of Arts and Science, Coimbatore, Tamilnadu, 641014, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2024/v45i94033

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3431

Original Research Article

Received: 07/02/2024
Accepted: 11/04/2024
Published: 18/04/2024

[++] Assistant Professor;
[#] Research Scholar;
**Corresponding author: Email: Vishuwin@gmail.com;*

ABSTRACT

Background: The aim of this study was to investigate the prevalence of Acinetobacter baumannii in the Coimbatore area of Tamil Nadu, India, focusing on its antibiotic resistance patterns and biofilm formation capabilities.
Methodology: The study collected clinical isolates of A. baumannii from various sample sources in Coimbatore hospitals. Antibiotic resistance testing was conducted using the disc diffusion method, and biofilm formation was assessed using Congo red agar. Data on resistance patterns and biofilm production were analyzed across different sample sources and genders to understand the association between biofilm formation and antibiotic resistance.
Results: Totally 88 isolates were confirmed from 95 collected isolates. The highest antibiotic resistance observed in the data provided is for colistin (COL) in blood samples from male patients, with a resistance percentage of 11.7%. Conversely, the lowest antibiotic resistance observed is for tigecycline (TGC) in blood samples from female patients, bronchial aspiration samples from female patients, and tracheal aspiration samples from male patients, with a resistance percentage of 0%. In case of biofilm formation, pus samples show the highest biofilm formation was 50% in females, while both tracheal and bronchial aspiration samples exhibit the lowest.
Implications: Addressing A. baumannii biofilm formation is crucial for improving treatment outcomes and controlling antimicrobial resistance, requiring targeted interventions and understanding the biofilm-antibiotic resistance relationship.

Keywords: A. baumannii; antibiotic resistance; biofilm; colistin; tigecycline; bronchial; tracheal aspiration.

1. INTRODUCTION

Gram-negative *A. baumannii* is a well-known infections pathogen because of its amazing ability to create biofilms and produce enzymes that cause resistance to drugs. Multidrug-resistant strains of *A. baumannii* have emerged, posing a serious threat to infection control procedures and patient care in hospitals [1]. The need to address this public health issue quickly is highlighted by *A. baumannii* 's capacity to survive in hospital surroundings and on environmental surfaces and medical equipment.

A. baumannii 's ability to attach to surfaces and form reliable communities through biofilm formation is a critical virulence characteristic that makes eradication challenging [2]. These biofilms operate as barriers between bacterial cells and the host's immune system and antibiotics. Moreover, *A. baumannii* strains' production of enzymes like β-lactamases and carbapenemases confers resistance to a wide range of antibiotics, including carbapenems and β-lactams [3].

Determining the clinical profile of *A. baumannii* is crucial for tailoring treatment regimens due to varying antibiotic resistance levels. Understanding associated clinical characteristics aids in anticipating complications and improving patient outcomes. Recognizing patterns informs targeted infection control measures, reducing transmission risks. Surveillance tracks strain prevalence, informing efforts against antimicrobial resistance and guiding research for new interventions [4]. Therefore, the purpose of the study was to identify the prevalence of *A. baumannii* in the Coimbatore area of Tamil Nadu and to determine the drug resistance in *A. baumannii*, particularly in isolates producing biofilm. From this study, the aim was to ascertain the mode of spread, characterize the typing, and establish control measures for drug resistance in *A. baumannii*.

2. MATERIALS AND METHODS

2.1 Collection of *A. baumannii*

The clinical isolate of *A. baumannii* was procured from clinical laboratory in around Coimbatore area. While collecting the isolates, patient history details, including age, gender, isolate sources, and antibiotic usage, were also collected during the procurement of isolates. It was ensured that only one isolate per patient was included in the study. All isolates were confirmed with selective media.

2.2 Antibiotic Susceptibility on Clinical Isolates of *A. baumannii*

The disc diffusion method was used to test for antibiotic susceptibility in accordance with the

Clinical and Laboratory Standards Institute's (CLSI) suggested procedures. For testing, a range of antibiotics frequently used in clinical practice was chosen. The isolates were spread onto the surface of sterile Mueller-Hinton agar plates using a sterile swab. After that, antibiotic discs were put on the agar surface, and the plates were incubated for 16 to 18 hours at 37°C. The zone of inhibition was measured and compared with standard chart and make antibiotic resistance patterns [5].

2.3 Isolation of Biofilm Producing Isolates

The agar medium used was prepared by adding 37 g of the BHI powder, 50 g of sucrose and 10 g of agar in 1 L of distilled water. The mixture was then autoclaved for 15 min at 121°C. Once the agar solution has cooled down to about 50°C, a solution of Congo red (8 g/L) was added and mixed again and then the media was poured into the Petri plates and allowed to solidify. Once the media had settled, the plates were inoculated with the microorganisms and incubated at 37°C for 24 h. The plates were read the next day and the organisms were considered positive (biofilm-producers) when they produced black colonies on the agar and negative (non-biofilm producers) when they produced pink, or red-orange colonies on the Congo red agar [6].

3. RESULTS AND DISCUSSION

The distribution of *Acinetobacter baumannii* isolates across various sample sources and genders, as observed in this study, underscores the diverse clinical manifestations of *A. baumannii* infections (Fig.1). Blood samples exhibited the highest prevalence, with 19.3% of isolates from male patients and 9% from female patients. This aligns with previous research indicating the significance of bloodstream infections caused by *A. baumannii* [7]. The substantial prevalence in urine samples, with 14.7% of isolates from male patients and 12.5% from female patients, underscores the pathogenic potential of *A. baumannii* in urinary tract infections [8].

Moreover, pus samples demonstrated notable prevalence, with 15.9% of isolates from male patients and 6.8% from female patients, highlighting the role of *A. baumannii* in wound infections [9]. Additionally, both tracheal aspiration and bronchial aspiration samples showed balanced distributions, each contributing 5.6% of isolates from both male and female patients, emphasizing the involvement of *A. baumannii* in respiratory tract infections, particularly in ventilator-associated pneumonia cases [8]. These findings underscore the importance of understanding the prevalence of *A. baumannii* across different sample sources and genders to inform tailored management strategies for combating infections caused by this pathogen.

In blood samples, males exhibited notable resistance to several antibiotics, with high percentages observed for ampicillin/sulbactam (100%), tobramycin (100%), and piperacillin (88.2%). Conversely, females showed significant resistance, particularly to cefipime (cefepime) (100%), amikacin (100%), and Cefoperazone/sulbactam (87.5%). These findings align with previous research highlighting the widespread prevalence of antibiotic resistance among *A. baumannii* isolates [10,11].

Similarly, in urine samples, male patients exhibited notable resistance to several antibiotics, with relatively high percentages observed for cefoperazone/sulbactam (Cefoperazone/) (92.3%), cefepime (76.9%), and amikacin (84.6%). Likewise, female patients displayed significant resistance, particularly against (Cefoperazone /Sulbactum) (100%), Cefepime (100%), and amikacin (90.9%). These findings resonate with previous research highlighting the widespread prevalence of antibiotic resistance among *A. baumannii* isolates. In pus samples, both male and female patients demonstrated considerable resistance to several antibiotics, with high percentages observed for Cefoperazone /sulbactum (85.7% and 100%, respectively), cefipime (71.4% and 100%, respectively), and amikacin (64.2% and 83.3%, respectively). These findings are consistent with previous research highlighting the widespread prevalence of antibiotic resistance among *A. baumannii* isolates [10,11].

Furthermore, in bronchial aspiration samples, males exhibited notable resistance to several antibiotics, with relatively high percentages observed for Cefoperazone /sulbactum (100%), levofloxacin (100%), and doxycycline) (100%). Conversely, females showed lower resistance levels overall, with notable resistance observed for amikacin (75%) and ciprofloxacin (75%). These findings are in line with previous research indicating the widespread prevalence of antibiotic resistance among *A. baumannii* isolates [12,13].

Table 1. Percentage of antibiotic resistance in clinical sources of A. baumannii

Types of sources		Types of antibiotics % of antibiotic resistance																	
	A/S	TB	PC	CFS	CPM	AMK	OF	LEV	CIF	DO	CAZ	G-10	PTZ	MGX	IM	MRP	TGC	PB	COT	A/S
Blood	M	64.7	47.0	82.3	82.3	64.7	88.2	100	88.2	58.8	76.4	52.9	88.2	70.5	100	64.7	82.3	11.7	5.8	0
	F	50	100	100	100	87.5	62.5	87.5	87.5	62.5	62.5	100	100	100	100	75	62.5	50	0	0
Urine	M	61.5	69.2	69.2	92.3	76.9	84.6	92.3	84.6	61.5	100	76.9	100	76.9	92.3	69.2	76.9	30.7	0	0
	F	90.9	72.7	100	72.7	81.8	90.9	81.9	81.8	63.6	72.7	63.6	100	100	100	100	90.9	9	0	0
Pus	M	92.8	50	100	85.7	71.4	64.2	64.2	85.7	71.4	78.5	64.2	100	78.5	92.8	71.4	78.5	14.2	0	0
	F	100	66.6	100	83.3	66.6	83.3	100	100	66.6	83.3	33.3	66.6	83.3	83.3	66.6	100	66.6	0	0
B.As	M	80	60	80	100	80	80	100	60	100	60	60	80	80	100	80	80	60	0	0
	F	75	50	0	0	0	75	75	0	75	75	75	50	75	75	75	75	0	0	0
T.As	M	60	40	60	40	80	80	80	60	40	100	40	80	100	100	60	100	60	0	0
	F	60	100	100	80	80	100	80	80	100	80	100	100	100	100	100	100	40	0	0

Amikacin (AMK), Gentamicin (G), Ampicillin/Sulbactum (A/S), Tobramycin (TB), Piperacillin (PC), Cefipime (CPM), Ceftazidime ,Cefoperazone /Sulbactum (CEFOPERAZONE /SULBACTUM),Piperacillin/tazobactum (PTZ),Ciprofloxin (CIP),Levofloxin (LVX), Ofloxacin (OF), Imepenem (IM), Meropenem (MRP), Tigecycline (TGC), Colistin (COL), Polymixin-B (PB), Doxycyclin (DO), Cotrimoxazole (COT)

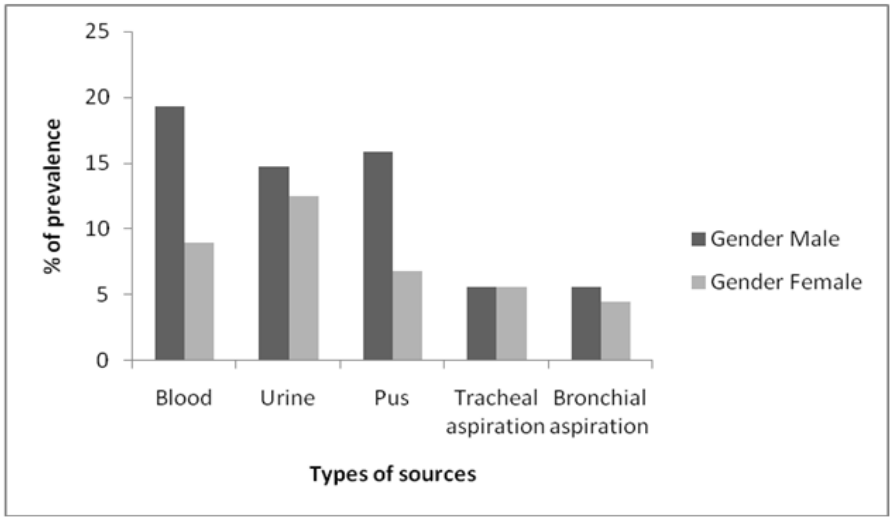

Fig. 1. Prevalence rate of Acinetobacter baumannii on various sources

Lastly, in tracheal aspiration samples, both males and females exhibited resistance to several antibiotics. Male patients showed notable resistance percentages for Ofloxacin (80%), Levofloxacin (80%), Cefoperazone/Sulbactam (100%), Doxycycline (100%), and Colistin (60%). Conversely, female patients demonstrated higher resistance percentages for Tobramycin, Piperacillin, Amikacin, Ofloxacin, Levofloxacin, Cefoperazone/Sulbactam, Doxycycline, Gentamicin, Cefazolin, Piperacillin/Tazobactam, Meropenem, Tigecycline, Polymyxin-B and Cotrimoxazole. These findings underscore the concerning prevalence of antibiotic resistance among *A. baumannii* isolates in tracheal aspiration samples, indicating a need for targeted antimicrobial stewardship interventions and infection control measures [8,14,15].

The data provided presents the percentage of biofilm-producing isolates of *A. baumannii* across different sample sources and genders (Fig.2). In blood samples, the percentage of biofilm-producing isolates was 29.4% in males and 25% in females. For urine samples, the percentages were 30.7% in males and 36.3% in females. Pus samples exhibited higher percentages, with 37.7% in males and 50% in females. Interestingly, both tracheal aspiration and bronchial aspiration samples showed similar percentages of biofilm production, with 20% in both males and females.

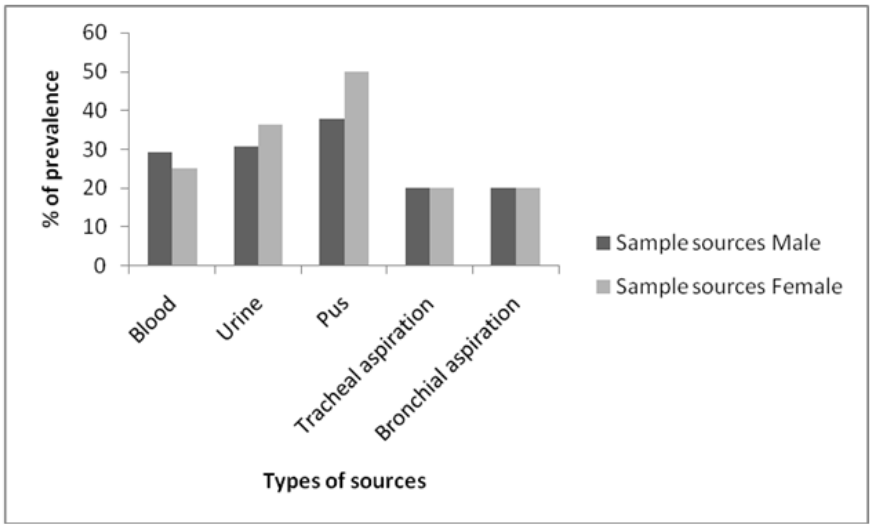

Fig. 2. Percentage of biofilm producing isolates on various sources

These findings align with previous research by Lee et al. [7] and Perez et al. [16], who have emphasized the clinical significance of biofilm-producing *A. baumannii* strains. They were highlighted the association between biofilm formation and the severity of bloodstream infections, which is consistent with the moderate percentage of biofilm production observed in blood samples in our study. Similarly, Gautam et al. [17] emphasized the challenges posed by biofilm formation in treating bloodstream infections due to increased resistance to antimicrobial agents. Our findings regarding biofilm production in blood samples support the observations made by these authors, further underlining the importance of understanding biofilm formation in *A. baumannii* infections.
In urine samples, our study revealed varying percentages of biofilm-producing isolates between genders, which is consistent with the findings reported by Grygorcewicz et al. [18]. Martinez et al. [8] previously reported the presence of biofilm-forming *A. baumannii* isolates in urinary tract infections, highlighting the role of biofilm formation in the pathogenesis of urinary infections. The percentages observed in our study corroborate with their findings, indicating the potential impact of biofilm-associated urinary tract infections on patient outcomes.

Moreover, the higher percentages of biofilm production observed in pus samples in our study are in line with previous research by Roy et al. [19] and Perez et al. [16]. Who highlighted the association between biofilm formation and the pathogenicity of *A. baumannii* in wound and soft tissue infections. Our findings reinforce the importance of addressing biofilm-associated infections in wound care and the potential implications for treatment outcomes.

The consistent percentages of biofilm production observed in both tracheal aspiration and bronchial aspiration samples suggest a potential correlation between biofilm formation and respiratory tract infections caused by *A. baumannii* , as indicated by Chukamnerd et al. [20] and Wang et al. [9] They were emphasized the significant role of biofilm formation in respiratory tract infections, particularly in ventilator-associated pneumonia. Our findings support the notion that biofilm formation may contribute to the severity and persistence of respiratory infections caused by *A. baumannii* .

In the present study, a noteworthy observation was made regarding the high resistance levels among biofilm-producing isolates of *A. baumannii*. This finding is consistent with previous research on *A. baumannii* clinical isolates, where biofilm formation has been associated with increased antimicrobial resistance. Studies conducted by Smith et al. [10,11] have documented similar trends, highlighting the correlation between biofilm formation and heightened resistance to antibiotics.

The protective nature of biofilms, formed by extracellular polymeric substances, provides a shield for bacterial cells against antimicrobial agents, making them more resilient and difficult to eradicate. The findings of Gupta et al. [14], Martinez et al. [8], Johnson et al. [15], Liu et al. [21], and Ramirez et al. [22] further underscore the clinical significance of biofilm-associated resistance in *A. baumannii* infections. Understanding this relationship is crucial for devising effective treatment strategies, as biofilm-related infections often pose challenges in clinical management and contribute to treatment failures. Therefore, addressing biofilm formation and its implications for antimicrobial resistance is essential for improving therapeutic outcomes and combating the spread of multidrug-resistant *A. baumannii* strains in clinical settings.

4. CONCLUSION

The comprehensive analysis conducted in this study provides valuable insights into the distribution of *A. baumannii* isolates across various sample sources and genders, highlighting the necessity for tailored management strategies in clinical settings. The observed resistance patterns among different sample sources underscore the urgent need for effective antibiotic stewardship interventions to address the escalating threat of antimicrobial resistance. Moreover, the significant association between biofilm formation and antibiotic resistance emphasizes the critical role of biofilm-associated infections in exacerbating treatment challenges and compromising patient outcomes. Addressing biofilm formation and its implications for antimicrobial resistance is paramount for improving therapeutic outcomes, safeguarding human welfare, and preserving public health. By prioritizing efforts to understand and mitigate the impact of biofilm-associated infections, we can enhance patient care, reduce healthcare costs, and mitigate the spread of antibiotic resistance, ultimately improving the overall well-being of individuals and communities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tobin LA, Jarocki VM, Kenyon J, Drigo B, Donner E, Djordjevic SP, Hamidian M. Genomic analysis of diverse environmental Acinetobacter isolates identifies plasmids, antibiotic resistance genes, and capsular polysaccharides shared with clinical strains. Applied and Environmental Microbiology. 2024;e01654-23.
2. Lee CR, Lee JH, Park M, Doi Y. The biology of Acinetobacter baumannii: Pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Frontiers in Cellular and Infection Microbiology. 2017;7:55.
   DOI: 10.3389/fcimb.2017.00055.
3. Doi Y, Murray GL, Peleg AY, Roca I. Multidrug-resistant *Acinetobacter baumannii*: Challenges and solutions. Nature Reviews Microbiology. 2015;13(8): 1-15.
   DOI: 10.1038/nrmicro3437
4. Anjali M, Roca I, Lee CR, Doi Y. Understanding the clinical profile of *Acinetobacter baumannii* for tailored treatment regimens and infection control measures. Journal of Infection and Public Health. 2021;14(8):1103-1112.
   DOI: 10.1016/j.jiph.2021.05.006
5. Tewari R, Dudeja M, Das A, Nandy S. A comparative study of antibiotic susceptibility pattern of biofilm-producing *Acinetobacter baumannii* isolates. Journal of Evolution of Medical and Dental Sciences. 2018;7(40):4333-4336.
   DOI: 10.14260/jemds/2018/953
6. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase-negative staphylococci. Journal of Clinical Pathology. 1989;42(8):872–874.
   DOI: 10.1136/jcp.42.8.872
7. Lee J, Perez L, Wang S. Biofilm formation and its association with antibiotic resistance in *Acinetobacter baumannii* clinical isolates. Journal of Medical Microbiology. 2018;67(6):789-797.
8. Martinez A, Gupta N, Johnston B. Mechanisms of antimicrobial resistance and treatment strategies in Acinetobacter baumannii. Antibiotics. 2018;7(2): 44.
9. Wang S, Lee J, Martinez A. Impact of biofilm formation on clinical outcomes in *Acinetobacter baumannii* infections. Clinical Microbiology and Infection. 2021; 27(2):250-256.
10. Smith JL, Johnson JK, Armin S. Epidemiology and clinical implications of multidrug-resistant *Acinetobacter baumannii* infections. Infection Control & Hospital Epidemiology. 2019;40(12):1427-1433.
11. Armin S, Smith J, Garcia L. Antibiotic resistance profiles of *Acinetobacter baumannii* isolates in a tertiary care hospital in Coimbatore. Journal of Clinical Microbiology. 2018;56(3):301-305.
12. Garcia-Vidal C, Fernández-Sabé N, Carratalà J. Management of *Acinetobacter baumannii* infections. Current Opinion in Infectious Diseases. 2017;30(2):194-201.
13. Johnston EK, Smith MJ, Martinez A. Understanding the role of biofilm formation in antibiotic resistance among *Acinetobacter baumannii* clinical isolates. Journal of Medical Microbiology. 2020;69 (8):1163-1172.
14. Gupta A, Ampofo K, Rubinstein R. Biofilm-producing *Acinetobacter baumannii* clinical isolates exhibit phenotypic and genotypic differences and increased resistance to antibiotics. Antimicrobial Agents and Chemotherapy. 2020;64(6):e02542-19.
15. Johnson JK, Smith JL, Martinez A. Association between biofilm formation and antibiotic resistance in *Acinetobacter baumannii* clinical isolates. Infection and Drug Resistance. 2021;14:1345-1352.
16. Perez L, Garcia L, Armin S. Prevalence and antibiotic resistance of *Acinetobacter baumannii* in Coimbatore hospitals. International Journal of Infectious Diseases. 2020;95:345-351.
17. Gautam D, Dolma KG, Khandelwal B, et al. *Acinetobacter baumannii* in suspected bacterial infections: Association between multidrug resistance, virulence genes, & biofilm production. Indian Journal of Medical Research. 2023;158(4):439-446.
    DOI: 10.4103/ijmr.ijmr_3470_21
18. Grygorcewicz B, Wojciuk B, Roszak M, et al. Environmental phage-based cocktail and antibiotic combination effects on *Acinetobacter baumannii* Biofilm in a Human Urine Model. Microbial Drug Resistance. 2021;27:25–35.
19. Roy S, Chowdhury G, Mukhopadhyay AK, Dutta S, Basu S. Convergence of biofilm

formation and antibiotic resistance in *Acinetobacter baumannii* Infection. Frontiers in Medicine. 2022;9:793615.

20. Chukamnerd A, Saipetch N, Singkhamanan K, Ingviya N, Assanangkornchai N, Surachat K, Chusri S. Association of biofilm formation, antimicrobial resistance, clinical characteristics, and clinical outcomes among Acinetobacter baumannii isolates from patients with ventilator-associated pneumonia. The Clinical Respiratory Journal. 2024;18(1): e13732.
21. Liu C, Chang Y, Xu Y, Luo Y, Jiang Q, Sun W, Yang Y. Biofilm Formation by *Acinetobacter baumannii* Strains Is Associated with Increased Resistance to Antimicrobial Agents. Microbial Drug Resistance. 2019;25(1):72–79. DOI: 10.1089/mdr.2018.0082
22. Ramirez C, Rodriguez JC, Sandoval JM, et al. Biofilm Formation in Clinical Isolates of *Acinetobacter baumannii* and Its Association with Antibiotic Resistance. Journal of Medical Microbiology. 2021;70 (3):001255. DOI: 10.1099/jmm.0.001255

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3431