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Original Article

Microbiology Section

Detection of Biofilm Formation among Drug-resistant *Acinetobacter* spp. Isolated from ICUs at a Tertiary Care Hospital: A Cross-sectional Study

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ABSTRACT

Introduction: *Acinetobacter spp.* has emerged as an important hospital-acquired and opportunistic agent due to its survival capability in adverse conditions, saprophytic presence, and increasing resistance to antimicrobials. The irrational use of antibiotics, along with biofilm formation, plays an important role in producing Extensively Drug-resistant (XDR) and Multidrug-Resistant (MDR) *Acinetobacter* species, especially *Acinetobacter calcoaceticus-baumannii complex (Acb complex),* in the hospital environment, contributing to morbidity and mortality.

Aim: To detect biofilm production among *Acinetobacter* species isolated from various clinical samples received from Intensive Care Units (ICUs) as well as their antibiotic sensitivity pattern.

Materials and Methods: A cross-sectional study was conducted in the Microbiology Department at Teerthanker Mahaveer Medical College and Research Centre, Moradabad, Uttar Pradesh, India from January 2022 to April 2023. Patients of all age groups and both genders were included in the study after obtaining informed consent. Clinical specimens, including endotracheal secretions, endotracheal tips, pus, urine, sputum, tissue, and other body fluids, were collected from ICUs where *Acinetobacter spp*. was detected. A total of 223 cases were included. The specimens were collected using clean, leak-proof, sterile containers with proper aseptic precautions. Antimicrobial susceptibility testing was performed according to the guidelines set by the Clinical and Laboratory Standards Institute (CLSI) in 2022 and identifies MDR and XDR strains. The biofilm production of isolates was determined using a quantitative adherence assay. The data generated was entered into Microsoft excel, and statistical analysis was conducted using International Business Machines (IBM) Statistical Package for Social Sciences (SPSS) statistical software version 28.0. The results were then presented as descriptive statistics.

Results: Among the 223 *Acinetobacter spp*. isolates, 159 (71.3%) were identified as Acb complex (*Acinetobacter calcoaceticus-baumannii complex*), followed by *A.lwofi* with 39 (17.5%) isolates and *A. haemolyticus* with 16 (7.2%) isolates. Acinetobacter showed resistance, in descending order of frequency, to amoxicillin+clavulanic acid (211 isolates, 94.6%), ciprofloxacin (211 isolates, 94.6%), trimethoprim-sulfamethoxazole (208 isolates, 93.3%), cefotaxime (198 isolates, 88.8%), cefepime (187 isolates, 83.8%), gentamycin (178 isolates, 79.8%), amikacin (152 isolates, 68.2%), piperacillin-tazobactam (68 isolates, 30.5%), imipenem (72 isolates, 32.3%), meropenem (71 isolates, 31.8%), polymyxin B (12 isolates, 5.4%), and colistin (2 isolates, 0.9%). The maximum antibiotic resistance was observed in *Acb complex*, with 208 (93.3%) strains being MDR producers and 32 (14.3%) strains being XDR producers. Biofilm production was observed in 214 isolates (95.9%), with 127 (56.9%) exhibiting strong biofilm production 63 (28.2%) showing moderate biofilm production, and 24 (10.8%) showing weak biofilm production. All MDR strains were found to produce biofilm, and out of those, 127 (61.1%) exhibited strong biofilm production. Among the XDR strains, all 32 (100%) were found to produce strong biofilm.

Conclusion: In conclusion, *Acinetobacter* spp. has a high propensity for developing MDR, and the formation of biofilms further aids the organism in surviving under strenuous conditions, making it difficult to treat. Therefore, regular surveillance of Hospital-acquired Infections (HAI), the prevention of misuse and overuse of antibiotics, prescribing antibiotics based on antibiogram patterns, formulating antibiotic policies, and implementing bundle care approaches for the prevention of HAI are crucial in preventing antibiotic resistance.

Keywords: Antibiotic resistance, Extensively drug-resistant, Intensive care unit, Multidrug-resistant

INTRODUCTION

Acinetobacter spp. is Gram-negative coccobacilli, non fermenters present throughout healthcare settings. They are opportunistic healthcare-associated pathogens, causing a wide range of infections such as Ventilator-associated Pneumonia (VAP), wound infections, Urinary Tract Infections (UTIs), peritonitis, bacteremia, and infections associated with indwelling devices, particularly in ICUs [1,2]. *Acinetobacter spp*. has emerged as a significant hospital-acquired and opportunistic agent due to its ability to survive in adverse conditions, its saprophytic presence, and its increasing resistance to antimicrobials [3]. Among the isolated species, *Acinetobacter baumannii*, considered a "Red alert pathogen," is the most common and exhibits a high level of resistance to major antibiotics. MDR in *Acinetobacter spp*. is attributed to carbapenemase production and biofilm formation [4,5]. The high antibiotic resistance observed may be linked to the overexpression of efflux pumps, reduced permeability, resistance islands containing gene clusters encoding for antibiotics, and carbapenemase production especially Carbapenem-hydrolysing class D β-Lactamases (CHDLs) [6].

Biofilm is a polymeric matrix produced by a structured community of microorganisms that adheres to inert or living surfaces [7,8]. Biofilm enables organisms to spread in hospital settings by attaching to various surfaces, such as central and peripheral line catheters, Central Nervous System (CNS) shunts, or Foley catheters [9]. It plays a key role in the transfer of antibiotic resistance via plasmids, transposons/integrons, and intercellular communication commonly known as quorum sensing [10]. Biofilm formation depends on various factors, including Biofilm-associated protein (Bap), Outer membrane protein A (OmpA), *CsuA/BABCDE* chaperone-usher pili assembly, among others [11]. Studies suggest that biofilm-producing

strains of *Acinetobacter spp*. exhibit significantly higher antimicrobial resistance compared to non biofilm producers [10]. Biofilm affects the efficacy of antimicrobial agents by reducing their penetration capability and trapping them within the exopolysaccharide matrix, acting as a protective mechanism that enables survival in different environmental conditions [10]. Therefore, the irrational use of antibiotics coupled with biofilm formation plays a significant role in the emergence of XDR and MDR *A. baumannii* in the hospital environment, contributing to morbidity, mortality, and the emergence of new antibiotic resistance phenotypes [12,13]. Pulsed-field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) are useful in detecting the genotypic relationships among *A.baumannii* strains in hospital settings [14].

Emphasising the importance of biofilm production and its role in MDR organisms, as well as evaluating biofilm production among drug-resistant isolates, is of paramount importance for effectively managing *Acinetobacter* species in HAIs. However, studies related to this topic are scarce, especially in this part of Northern India. The hypothesis of the present study was that there is a direct association between biofilm production in clinical isolates and drug resistance.

The present study aimed to detect and compare biofilm production among drug-resistant Acinetobacter species isolated from various clinical samples obtained from ICUs. The primary objective of the study was to determine the antibiotic sensitivity pattern of these isolates, while the secondary objective was to detect the production of biofilms among drug-resistant strains, such as MDR and XDR.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Microbiology Department at Teerthanker Mahaveer Medical College and Research Centre, Moradabad, Uttar Pradesh, India from January 2022 to April 2023. The study received approval from the central research committee, and ethical clearance was obtained from the Ethical Committee (TMMC& RC/IEC/20-21/119).

Inclusion criteria: Patients of all age groups and both genders were included in the study after obtaining informed consent. All clinical specimens, including endotracheal secretions, endotracheal tips, pus, urine, sputum, tissue, and other body fluids, collected in clean, leak-proof, sterile containers with proper aseptic precautions received from ICU where *Acinetobacter spp* was detected, were included in the present study. Blood samples were collected in automated BactAlert bottles.

Exclusion criteria: Isolates other than Acinetobacter spp. and growth occurring in a mixture of organisms were excluded from the study.

Sample size calculation: The required sample size (N) was determined using the formula $N=Z^2 P(1-P)/d^2$, where 'CI' is the confidence interval (95%), d is the margin of error (5%), 'P' is the prevalence (9.3%) [15] and 'Z' is 1.96 for a 95% CI. The calculated minimum sample size was 153.49, rounded up to 154. However, the authors included 223 isolates as we received more isolates during the study period.

Study Procedure

A 5% defibrinated sheep blood agar and MacConkey's agar plates were used to inoculate the specimens, which were then incubated aerobically at 35°C for 24 hours. Isolate identification was based on standard laboratory techniques, and different *Acinetobacter* spp. were identified using the automated Vitek 2 Compact system. Only *Acinetobacter* spp. was further processed for antibiotic sensitivity testing and biofilm formation. Antibiotic susceptibility testing was performed using the modified Kirby-Bauer disc diffusion method according to CLSI standards, 2022 [16]. MDR isolates were defined as those resistant to at least one agent in ≥3 classes of the tested antimicrobials, while XDR strains were resistant to at least one agent in all classes but two or fewer antimicrobial categories [17].

Biofilm production of the isolates was detected using a quantitative adherence assay. In Trypticase Soy Broth (TSB), isolates were inoculated and incubated at 37°C for 16-24 hours. In 96-well microtiter plates, 2 µL of the suspension was mixed with 198 µL of TSB and incubated at 37°C for 24 hours. After washing thrice with 200 µL of Phosphate-Buffered Saline (PBS), the plates were stained with 50 µL of 0.1% crystal violet. After another three washes with 200 µL of distilled water, 200 µL of 5% isopropanol was added. The Optical Density (OD) at 570 nm was determined using a microtitre plate reader. Positive and negative controls, ATCC-27853 *P.aeruginosa* and ATCC-25923 *S.aureus*, respectively, were included. All isolates along with positive and negative control were tested in triplicate and the average OD was determined. By definition, OD cut-off (ODc) was determined by taking an average OD of negative control+(3×SD of negative control). Biofilm production was interpretated as non biofilm producer: OD≤ODc, Weak biofilm producer: ODc<OD≤2×ODc, medium biofilm producer: 2×ODc<OD≤4×ODc and strong biofilm producer: 4×ODc<OD [18].

STATISTICAL ANALYSIS

The data generated was entered into Microsoft excel, and statistical analysis was conducted using IBM SPSS statistical software version 28.0. Afterwards, the data was presented as descriptive statistics.

RESULTS

During the study period, a total of 223 Acinetobacter spp. were identified from various clinical samples collected. Among them, 139 (62.3%) were males and 84 (37.6%) were females, resulting in a ratio of 2:1. The most commonly affected age group was 51-60 years, with 77 cases (34.5%), followed by the 61-70 years group with 54 cases (24.2%), as illustrated in [Table/Fig-1].

Age group (years)	No. of isolates	Males	Females
0-10	17 (7.6%)	11	6
11- 20	04 (1.8%)	2	2
21- 30	11 (4.9%)	7	4
31-40	18 (8.1%)	12	6
41-50	29 (13%)	18	11
51-60	77 (34.5%)	43	34
61-70	54 (24.2%)	38	16
>70	13 (5.8%)	8	5
Total	223	139 (62.3%)	84 (37.6%)

[Table/Fig-1]: Age and sex-wise distribution of *A.baumannii* isolated from various samples.

Among the 223 clinical isolates, 159 (71.3%) were identified as *Acinetobacter calcoaceticus-baumannii complex (Acb complex)*, which was the predominant species. This was followed by 39 isolates (17.5%) of *A.lwofi*, 16 (7.2%) of *A.haemolyticus*, 6 (2.7%) of *A.junii*, *and 3 (1.3%) of A.radioresistens*, as displayed in [Table/Fig-2].

In the present study, *Acinetobacter spp*. was most commonly isolated from Endotracheal (ET) secretion and ET tube samples, accounting for 93 cases (41.7%). Sputum samples accounted for 36 cases (16.1%), while blood and central line samples accounted for 32 cases (14.3%). Urine samples accounted for 30 cases (13.4%), and other sample types were also included, as depicted in [Table/Fig-2].

Maximum antibiotic resistance was found in the *Acb complex*, followed by *A.lwoffi* and *A. haemolyticus*, as shown in [Table/Fig-3]. In the present study, 208 (93.3%) strains were MDR, and 32 (14.3%) strains were XDR, as indicated in [Table/Fig-3]. Among the 223 *Acinetobacter spp*., 159 (71.3%) were Acb *complex (Acinetobacter calcoaceticus-baumannii complex),* followed by *A.lwofi* with 39 (17.5%) strains and *A.haemolyticus* with 16 (7.2%) strains. Acinetobacter demonstrated

Clinical samples	No. of isolates	Acb complex	*A.lwofii*	*A. haemolyticus*	*A.junii*	*A. radioresistens*
Sputum	36 (16.1%)	21 (13.2%)	9 (23.1%)	3 (18.7%)	2 (33.3%)	1 (33.3%)
ET secretions, ET tube, BAL fluid etc.	93 (41.7%)	79 (49.7%)	8 (20.5%)	5 (31.2%)	1 (16.7%)	0
Urine	30 (13.4%)	18 (11.3%)	7 (17.9%)	3 (18.7%)	1 (16.7%)	1 (33.3%)
Blood and central line	32 (14.3%)	21 (13.2%)	7 (17.9%)	2 (12.5%)	1 (16.7%)	1 (33.3%)
Pus and tissue samples	21 (9.4%)	16 (10.1%)	4 (10.2%)	1 (6.2%)	0	0
*CSF and body fluids	11 (4.9%)	4 (2.5%)	4 (10.2%)	2 (12.5%)	1 (16.7%)	0
Total	223	159 (71.3%)	39 (17.5%)	16 (7.2%)	6 (2.7%)	3 (1.3%)

[Table/Fig-2]: Distribution of various *Acinetobacter spp*. in clinical samples (N=223).
*BAL fluid: Bronchoalveolar lavage; ET: Endotracheal; CSF: Cerebrospinal fluid; **Acb complex: *Acinetobacter calcoaceticus-baumannii complex; A. lwofii: Acinetobacter lwofii; A. haemolyticus: Acinetobacter haemolyticus; A. junii: Acinetobacter junii; A. radioresistens: Acinetobacter radioresistens*

Antibiotics	Resistant isolates	Acb complex (n=159)	*A.lwofii* (n=39)	*A. haemolyticus* (n=16)	*A.junii* (n=6)	*A. radioresistens* (n=3)
AC	211 (94.6%)	157 (98.7%)	36 (92.3%)	13 (81.2%)	3 (50%)	2 (66.7%)
G	178 (79.8%)	135 (84.9%)	31 (79.5%)	9 (56.2%)	2 (33.3%)	1 (33.3%)
Ak	152 (68.2%)	121 (76.1%)	22 (56.4%)	8 (50%)	1 (16.7%)	0
Co	208 (93.3%)	156 (98.1%)	35 (89.7%)	12 (75%)	3 (50%)	2 (66.7%)
Cf	211 (94.6%)	157 (98.7%)	36 (92.3%)	13 (81.2%)	3 (50%)	2 (66.7%)
Ce	198 (88.8%)	154 (96.8%)	32 (82%)	8 (50%)	3 (50%)	1 (33.3%)
Cpm	187 (83.8%)	148 (93.1%)	29 (74.3%)	7 (43.7%)	2 (33.3%)	1 (33.3%)
PT	68 (30.5%)	57 (35.8%)	9 (23.1%)	1 (6.2%)	1 (16.7%)	0
Mr	71 (31.8%)	60 (37.7%)	8 (20.5%)	3 (18.7%)	0	0
Imp	72 (32.3%)	60 (37.7%)	8 (20.5%)	3 (18.7%)	1 (16.7%)	0
PB	12 (5.4%)	10 (6.3%)	2 (5.1%)	0	0	0
CL	2 (0.9%)	2 (1.2%)	0	0	0	0
MDR strains	208 (93.3%)	156 (98.1%)	35 (89.7%)	12 (75%)	3 (50%)	2 (66.7%)
XDR strains	32 (14.3%)	29 (18.2%)	3 (7.7%)	0	0	0

[Table/Fig-3]: Antimicrobial resistance profile among *A. baumannii* isolates.
*AC: Amoxycillin+Clavulanic acid; G: Gentamycin; Ak: Amikacin; Co: Trimethoprim-sulfamethoxazole; Cf: Ciprofloxacin; Ce: Cefotaxime; Cpm: Cefepime; PT: Piperacillin/tazobactam; Mr: Meropenem; Imp: Imipenem; PB: Polymixin B; CL: Colistin; MDR: Multidrug-resistant; XDR: Extensively drug-resistant; **Acb complex: *Acinetobacter calcoaceticus-baumannii complex; A. lwofii: Acinetobacter lwofii; A. haemolyticus: Acinetobacter haemolyticus; A. junii: Acinetobacter junii; A. radioresistens: Acinetobacter radioresistens*

resistance, in descending order of frequency, to the following antibiotics: amoxicillin+clavulanic acid with 211 (94.6%), ciprofloxacin with 211 (94.6%), trimethoprim sulfamethoxazole with 208 (93.3%), cefotaxime with 198 (88.8%), cefepime with 187 (83.8%), gentamycin with 178 (79.8%), amikacin with 152 (68.2%), piperacillin-tazobactam with 68 (30.5%), imipenem with 72 (32.3%), meropenem with 71 (31.8%), polymyxin B with 12 (5.4%), and colistin with 2 (0.9%). Additionally, 208 (93.3%) strains were MDR producers, and 32 (14.3%) strains were XDR producers.

Biofilm production was observed in 214 isolates (95.9%). Among them, strong biofilm was detected in 127 strains (56.9%), while moderate and weak biofilm production were seen in 63 (28.2%) and 24 (10.8%) strains, respectively. It was found that all MDR strains were capable of producing biofilm, with 127 (61.1%) of them exhibiting strong biofilm production. Moderate and weak biofilm production were observed in 62 (29.8%) and 19 (9.1%) MDR strains, respectively. In contrast, among non MDR strains, 9 (60%) did not produce biofilm, while 5 (33.3%) and 1 (6.7%) strains showed weak and moderate biofilm production, respectively. None of the non-MDR strains were found to produce strong biofilm [Table/Fig-4]. Interestingly, all XDR strains were found to produce strong biofilm. Among non XDR strains, 95 (49.7%), 63 (32.9%), 24 (12.6%), and 9 (4.7%) displayed strong, moderate, weak, and no biofilm production, respectively [Table/Fig 4].

Biofilm production	Strong	Moderate	Weak	Non producer
MDR strains (n=208)	127 (61.1%)	62 (29.8%)	19 (9.1%)	0 (0%)
Non-MDR strains (n=15)	0 (0%)	1 (6.7%)	5 (33.3%)	9 (60%)
XDR strains (n=32)	32 (100%)	0 (0%)	0 (0%)	0 (0%)
Non XDR strains (n=191)	95 (49.7%)	63 (32.9%)	24 (12.6%)	9 (4.7%)

[Table/Fig-4]: Biofilm production among drug-resistant Acinetobacter strains.
MDR: Multidrug-resistant; XDR: Extensive drug-resistant

DISCUSSION

Acinetobacter is a notorious healthcare-associated pathogen, especially in ICUs, due to the usage of invasive devices and prolonged stays. Biofilm formation is considered an important virulence factor for its survival and the development of MDR strains. In the present study, the predominant species was the *Acb complex* (71.3%), followed by *A. lwofi* (17.5%) and *A. haemolyticus* (7.2%). This finding is comparable to studies conducted by Kumari M et al., where out of a total of 324 isolates, 167 (51.5%) were *Acinetobacter calcoaceticus-baumannii complex (Acb complex)*, followed by 83 (25.6%) *A. lwoffii*, 38 (11.7%) *A.haemolyticus*, 30 (9.3%) *A.radioresistens*, and 6 (1.9%) *A.junii* [19]. Gupta N et al., also reported similar results, with Acb complex accounting for 80 (72%) of total *Acinetobacter* isolates, and non Acb complex species {*Acinetobacter* lwoffii 16 (14%), *Acinetobacter haemolyticus* 13 (12%), *Acinetobacter junii* 1 (1%), *Acinetobacter radioresistans* 1 (1%)} comprising the remaining isolates [20]. This observation suggests that the Acb complex has a better survival mechanism even under stringent conditions.

In the present study, only ICU patients were considered, as they require prolonged hospitalisation, invasive devices, and treatment with multiple antibiotics, thereby providing a favourable environment for the colonisation and survival of *Acinetobacter* in a hospital set-up. The development of antibiotic resistance is associated with high morbidity and mortality in hospitalised patients, particularly in ICU settings [21,22]. The most common isolates in present study were obtained from ET secretion and ET tubes (41.7%), followed

by sputum (16.1%), blood and central line (14.3%), and urine (13.4%). These findings are in accordance with a study by Bala M et al., where the maximum isolation was from endotracheal aspirate (42%), followed by sputum (29%), pus (16%), blood and other sterile body fluids (6%), urine (4%), and bronchoalveolar lavage (3%) [23]. ET tubes, central lines, and urinary catheters are more prone to biofilm production, which protects them from antimicrobials and host defense mechanisms [24].

The high level of resistance to common antibiotics is due to *Acinetobacter*'s ability to acquire resistance easily, and biofilm formation supports the spread of resistance, making it a difficult-to-treat pathogen in the current period. In the late 1990s, carbapenems were the treatment of choice for *Acinetobacter* infection, but now carbapenem resistance is developing worldwide, causing serious concern [25]. *Acinetobacter* exhibited resistance, in descending order of frequency, to amoxicillin+clavulanic acid (94.6%), ciprofloxacin (94.6%), trimethoprim sulfamethoxazole (93.3%), cefotaxime (88.8%), cefepime (83.8%), gentamycin (79.8%), amikacin (68.2%), and piperacillin-tazobactam (30.5%). In the present study, the resistance to imipenem and meropenem was 32.3% and 31.8%, respectively. However, the prevalence of resistance to polymyxin B and colistin was 5.4% and 0.9%, respectively, making it the only antibiotic useful in the treatment of these resistant strains. In the present study, 93.3% and 14.3% of the strains were identified as MDR and XDR, respectively. The resistance pattern observed was similar to studies conducted by Shrestha M and Khanal B, where resistance patterns to various drugs included meropenem (19%), piperacillin (96%), piperacillin-tazobactam (43%), amikacin (51%), ceftazidime (84%), ceftriaxone (66%), co-trimoxazole (58%), gentamycin (57%), ciprofloxacin (55%), and tetracycline (53%). Additionally, eleven isolates of *Acinetobacter* were resistant to meropenem [22]. Another study by Monfared AM et al., showed that resistance to many antibiotics was over 90%, with sensitivity limited to colistin. Out of 118 isolates, nine were resistant to colistin [26].

Biofilm production is responsible for causing delayed drug diffusion through the biofilm matrix, altered growth rate of biofilm organisms, and other physiological changes due to the biofilm mode of growth [27]. In the present study, biofilm production was observed in 95.9% of isolates. Strong biofilm was detected in 56.9% of the isolates, while moderate and weak biofilm production was observed in 28.2% and 10.8% of isolates, respectively. These findings differ from another study where biofilm formation was reported in 73.7% of isolates [28]. The difference could be attributed to the fact that the present study focused on samples obtained specifically from ICUs where device usage is more common and biofilm formation is frequently observed.

All MDR strains were found to be producing biofilm, with 61.1% of them exhibiting strong biofilm production. Similarly, all XDR strains were found to produce strong biofilm. These results are consistent with another study from Tehran, where 92% of biofilm-forming strains were reported to be MDR and 86% were XDR [28]. It is crucial to develop new strategies that focus on inhibiting biofilm formation and eradicating preformed biofilms in order to combat biofilm-associated infections. Preventive strategies for biofilm production can include the use of: a) antibacterial polymers like silver nanoparticles or hydrogel matrices on medical devices, as well as; b) surface coatings with antibiofilm agents to inhibit biofilm formation by *A. baumannii* on medically relevant surfaces. Strategies for the eradication or dispersal of preformed biofilms in chronic infections can involve the; c) degradation of the biofilm matrix using enzymes or natural/synthetic compounds; d) targeting the quorum sensing mechanism of *A.baumannii* through Quorum Sensing (QS) inhibition or quorum quenching, and utilising; e) emerging therapeutic strategies such as phage therapy, photodynamic therapy, and nanoparticle-based therapy [29]. Hence, the findings of the present study contribute to the further management of these drug-resistant strains. Additionally, preventing biofilm formation can be achieved by implementing appropriate infection control measures such as environmental surveillance and cleaning, contact precautions, restricted use of broad-spectrum antibiotics, and adherence to proper guidelines for antibiotic usage.

Limitation(s)

In the present study, only *Acinetobacter* spp. was included. However, it is important to note that biofilm formation is a common virulence mechanism for other bacterial agents as well. Additionally, biofilm formation is more commonly observed in infections associated with invasive devices. Therefore, further studies are needed to better understand the process of biofilm formation in bacterial infections.

CONCLUSION(S)

Acinetobacter spp. have a high propensity for developing MDR, and biofilm formation further aids the organism in surviving in strenuous conditions, making it even more difficult to treat. The current treatment strategy for biofilm-associated infections depends on whether contaminated medical implants are involved or if the bacteria have directly colonised host tissues. Infections associated with indwelling medical devices often require the removal of the implant for successful treatment outcomes. In other cases where bacteria directly colonise host tissues are chronic infections. In such cases, reducing the biofilm through antibiotic treatment is currently the only feasible option. Therefore, regular surveillance of HAI, early removal of medical devices, avoiding misuse and overuse of antibiotics, prescribing antibiotics based on antibiogram patterns, formulating antibiotic policies, and implementing a bundle care approach to prevent HAIs are some of the key measures for preventing antibiotic resistance.

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